Actin-Dependent Regulation of *Borrelia burgdorferi* Phagocytosis by Macrophages

Xenia Naj and Stefan Linder

Abstract The spirochete *Borrelia burgdorferi* is the causative agent of Lyme disease, a multisystemic disorder affecting primarily skin, nervous system, and joints. If an infection with *Borrelia* proceeds unchecked, the disease can also enter a chronic stage, leading to the development of neuroborreliosis or cardiac arrhythmia. Successful elimination of *B. burgdorferi* by the host immune system is thus decisive for the positive outcome of a respective infection. Accordingly, host immune cells such as macrophages and dendritic cells have to be able to efficiently internalize and degrade infecting spirochetes. These processes are based on closely controlled rearrangements of the actin cytoskeleton, which enables the spatiotemporally fine-tuned formation of cellular protrusions and compartments that assist in the capturing, immobilization, and uptake of borreliae, as well as their further intracellular processing. Here, we discuss actin-based structures, in particular filopodia and coiling pseudopods that are involved in phagocytosis of *B. burgdorferi* by macrophages, their regulation by actin-associated proteins such as formins and Arp2/3 complex, as well as the subsequent intracellular processing of borreliae.

X. Naj · S. Linder (✉)
Institute for Medical Microbiology, Virology and Hygiene,
University Medical Center Eppendorf, 20246 Hamburg, Germany
e-mail: s.linder@uke.de

X. Naj
e-mail: xenia.naj@polytechnique.edu

X. Naj
Ecole Polytechnique, Laboratory of Biochemistry,
CNRS UMR 7654, 91128 Palaiseau Cedex, France

Current Topics in Microbiology and Immunology
DOI 10.1007/82_2016_26

Contents

1 Introduction

Borrelia burgdorferi is the causative agent of Lyme disease, a multisystemic disorder affecting primarily skin, nervous system, and joints. *Borrelia* belongs to the phylum of spirochetes, is characterized by a double membrane and an elongated helical morphology (Li et al. 2000), and can grow to lengths of 10–40 μm (Aberer and Duray 1991). Typical for spirochetes, borreliae feature a set of flagellae that run lengthwise along the cell body, in the periplasmic space between the inner and outer membrane, and enable considerable motility of the bacterium, with velocities of up to 4 μm/sec (Goldstein et al. 1994; Moriarty et al. 2008).

Borrelia burgdorferi sensu stricto is part of the *B. burgdorferi* sensu lato complex, which also encompasses further genospecies such as *Borrelia afzelii*, *Borrelia garinii* and others, many of which are pathogenic to humans. Borreliae typically propagate in rodents, deer, or birds and are transmitted by ticks of the *Ixodidae* family, with humans being inadvertent hosts (Lane et al. 1991). Once borreliae are transmitted by a blood meal, they can disseminate throughout the skin, which is often accompanied by the formation of *Erythema migrans*, a prominent rash that spreads from the center of infection and is enriched in neutrophils, dendritic cells, and macrophages (Salazar et al. 2003). These cells represent the first line of the host innate immune system, and their interaction with infecting borreliae is thus decisive for the outcome of a respective infection. In particular, uptake and elimination of borreliae by macrophages has been shown to be crucial to prevent dissemination of borreliae (Carrasco et al. 2015). Conversely, if an infection with *B. burgdorferi* proceeds unchecked, Lyme disease can also enter a chronic stage, leading to the development of neuroborreliosis or cardiac arrhythmia.

Successful uptake and elimination of infecting borreliae by macrophages involves a succession of tightly choreographed steps that are based on fine-tuned restructuring of the cytoskeleton, in particular of actin microfilaments. Macrophages form several actin-based structures during capturing and uptake of borreliae (Figs. 1 and 2). Molecular regulators of these structures, and particularly actin-regulatory factors such as formins and Arp2/3 complex, have been shown to critically influence effective phagocytosis of borreliae (Linder et al. 2001;

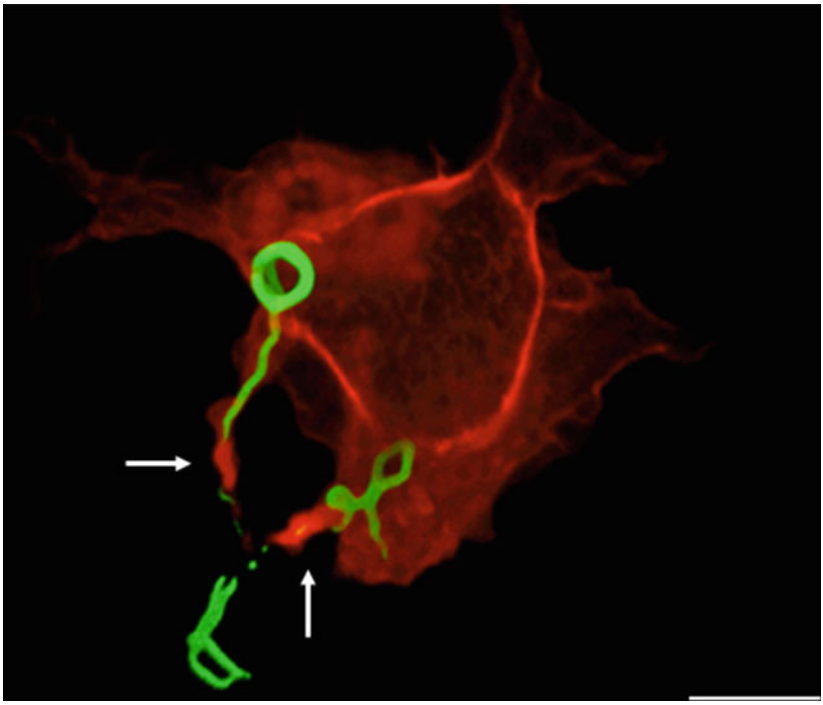

Fig. 1 Uptake of borreliae by human macrophages involves dynamic restructuring of the host actin cytoskeleton. Still image from confocal time-lapse video showing a primary human macrophage expressing RFP-Lifeact (*red*) internalizing several GFP-expressing spirochetes (*green*) with actin-rich cell protrusions (*white arrows*). Video can be openly accessed at http://www.linderlab.de/movies. Scale bar: 10 μm

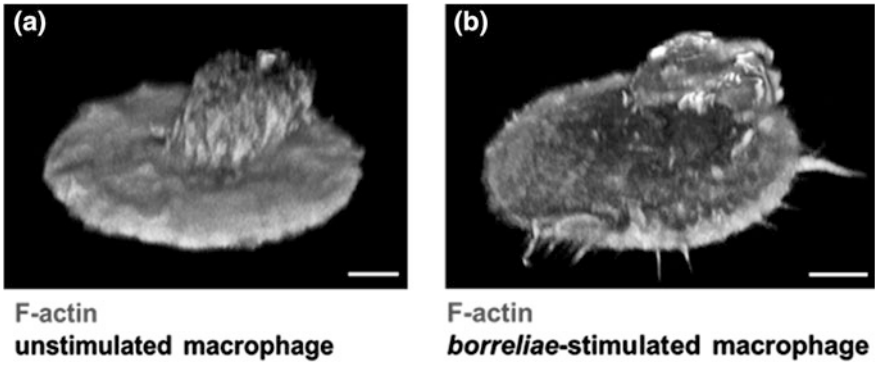

Fig. 2 *Borrelia* induces filopodia formation in human macrophages. Z-stacks of primary macrophages (stained for F-actin) without stimulation (**a**) or after 1 h of coincubation with *Borrelia burgdorferi* (**b**). Note formation of several filopodial protrusions after coincubation of macrophage with borreliae in (**b**). Scale bar: 5 μm

Hoffmann et al. 2014; Naj et al. 2013). Subsequently, regulators of vesicular trafficking such as small GTPases of the Rab family, steer the internalized spirochetes towards a degradative compartment (Naj and Linder 2015). Here, we discuss the current knowledge about actin-based uptake structures formed by macrophages during phagocytosis of borreliae, the intracellular processing of internalized spirochetes, as well as the respective molecular regulators of these processes.

2 *Borrelia* and the Stages of Lyme Disease

Lyme disease, also known as Lyme borreliosis, was first described in 1976 in Lyme, Connecticut, where an epidemic of juvenile rheumatoid arthritis occurred (Steere et al. 1977). In 1982, Willy Burgdorfer isolated spirochete bacteria from ticks of the *Ixodes* complex, which were abundant in this area. After the successful cultivation of the same spirochetes from patients, the causative agent of Lyme disease was called, based on its discoverer, *B. burgdorferi* (Burgdorfer et al. 1982).

To date, 18 different *Borrelia* genospecies are known (Margos et al. 2011). They are commonly referred to as the *B. burgdorferi* sensu lato complex. Among these 18 genospecies, seven have been identified to be infectious for humans, with three species being most frequently detected in patients. While *B. burgdorferi* sensu stricto is the most prevalent genospecies causing Lyme disease in North America, *Borrelia afzelii* and *Borrelia garinii* are the most commonly isolated human pathogenic species in Europe and Asia.

Lyme disease is a multisystemic disease that is considered to mainly originate from inflammatory responses to the *Borrelia* infection. The progression of Lyme disease is divided into three stages: (i) the early localized infection, (ii) the early disseminated infection, and (iii) the persistent infection (Zajkowska et al. 2012). However, not all three stages become necessarily apparent during the course of an infection.

The early, localized infection typically starts one to four weeks after transmission of borreliae with a painless skin rash spreading from the tick bite in a characteristic double ring shaped morphology called *Erythema migrans* (EM). EM develops through the response of immune cells, such as macrophages, neutrophils, and dendritic cells, which secrete inflammatory cytokines concomitantly with the spreading of the bacteria within the skin (Steere et al. 1983). Early infection with borreliae is often accompanied by flu-like symptoms, such as fever, malaise, and headache. If Lyme disease is diagnosed during that stage, successful treatment with antibiotics such as doxycycline or amoxicillin has a good prognosis (Jares et al. 2014). However, considering that EM occurs only in $\sim$60–80 % of all cases, infection with borreliae remains often unrecognized and can thus progress into the stage of the early disseminated infection.

During early disseminated infection, borreliae transmigrate from the skin into blood vessels, from where they spread through the blood stream to various organs (Kumar et al. 2015; Petzke and Schwartz 2015). Depending on the site of *Borrelia*

dissemination, the infection results in different symptoms. Therefore, beyond the originally identified manifestation in joints in the form of rheumatoid arthritis (Steere et al. 1977), patients can also develop cardiac symptoms like arrhythmia, skin lesions known as *Acrodermatitis atrophicans*, or Neuroborreliosis, which is accompanied by symptoms like facial palsy, meningitis, and encephalitis (Zajkowska et al. 2012; Steere et al. 2004).

Also at this later stage, patients can be cured by a prolonged treatment with antibiotics. However, in many cases symptoms persist even beyond such a regime. This stage is referred to as post-Lyme disease. It is under debate whether the symptoms are based on the presence of bacteria that persist despite the antibiotics therapy necessitating further or additional courses of antibiotics treatment (Aguero-Rosenfeld and Wormser 2015). The more widely accepted assumption, however, is that these posttreatment symptoms occur even in the absence of any remaining borreliae and are rather a consequence of damaged tissue, or of ongoing inflammation processes and autoimmune disorders (Berende et al. 2010; Pearson 2014).

To clearly answer the question whether disseminated borreliae are able to persist despite prolonged antibiotics treatment, successful cultivation of spirochetes from patient samples would be necessary. However, this is very inefficient and thus not reliable. Therefore, post-Lyme disease is still not well understood, and a clear definition of the causes as well as specific diagnostic tools are missing. The published guidelines of the "Infectious Diseases Society of America" (IDSA) advise against repeating courses of antibiotics treatment if symptoms reappear after a first antibiotic course (Wormser et al. 2006). Still, some uncertainty remains, as several studies detected persistent bacteria despite a long-term treatment with antibiotics (Stricker and Johnson 2013; Berndtson 2013). In rhesus macaques, which were infected with borreliae, low numbers of intact spirochetes were successfully recovered, despite an aggressive long-term antibiotics treatment (Embers et al. 2012). Due to these controversies, the existence of a post-Lyme syndrome, whether it results from a persistent infection, as well as its potential treatment, are still points of debate (Borgermans et al. 2014; Aguero-Rosenfeld and Wormser 2015).

3 Phagocytic Uptake of *Borrelia* by Macrophages

At the stage of the early localized infection, the immune system of the host can still prevent the spreading of bacteria. Thus, interaction of *Borrelia* with cells of the immune system, and especially with phagocytes, is critical for the outcome of the infection. EM biopsies have shown that T cells, macrophages and dendritic cells locally infiltrate the skin (Ziuzia Iu et al. 1999; Salazar et al. 2003; Duray 1989). In this review, we focus in particular on the interactions of *Borrelia* with macrophages, which are professional phagocytes and capable to efficiently eliminate bacteria from infected tissue.

Macrophages are part of the innate immune system, the first line of defense against infecting pathogens. At the same time, they also play a role as activators of the adaptive immune system. As professional phagocytes, macrophages are able to take up and efficiently eradicate a large number of bacteria per individual cell, through a process called phagocytosis. Phagocytosis is defined as the uptake of a particle >0.5 μm in diameter. It is a multistep process that involves detection of a phagocytic target, its capturing or immobilization, with subsequent internalization, followed by intracellular degradation.

Usually, recognition of bacteria as phagocytic targets takes place through either deposited opsonins, including factors of the complement system and antigen-targeting antibodies, or through conserved surface exposed proteins, so-called pathogen-associated molecular patterns (PAMPs). Opsonins and PAMPs are recognized by several cell surface receptors. Once a ligand–receptor interaction is established and the bacteria immobilized, macrophages develop localized protrusions, which engulf and help to internalize the pathogen. These steps of immobilization and internalization require a highly fine-tuned and localized regulation of the actin cytoskeleton. In the case of *Borrelia,* it is known that their phagocytosis is mediated by several different receptors, including opsonic receptors like FcγR (Benach et al. 1984; Montgomery et al. 1994) and the complement receptor 3 (CR3) (Garcia et al. 2005; Hawley et al. 2012; Cinco et al. 1997). Furthermore, internalization of borreliae can also be mediated by the non-opsonic toll like receptor 2 (TLR2) (Salazar et al. 2009), with downstream signaling involving both myeloid differentiation factor 88 (MyD88)-dependent but also -independent pathways. (Shin et al. 2009). It has been shown that knock-out mice, which are not able to express either TLR2, Fc receptor common gamma chain (FcεRγ) or CR3 develop higher *Borrelia* burdens and more pronounced symptoms (Wang et al. 2004; Lawrenz et al. 2003; Liu et al. 2004). In vitro, both opsonized and unopsonized borreliae were shown to attach to macrophages. However, opsonization of the spirochetes by serum containing factors of the complement system, or by *Borrelia*-targeting antibodies, enhances their attachment to macrophages 4–5 fold (Linder et al. 2001). Collectively, *Borrelia* phagocytosis is mediated by several receptors, which act in concert to allow efficient clearance of spirochetes.

Internalization of pathogens is accompanied by their uptake into a specific intracellular compartment, the phagosome, whose coat is derived from the pathogen-engulfing membrane, which is closed upon final internalization and pinched off from the plasma membrane (Fairn and Grinstein 2012). Phagosomes then undergo a process of maturation, which is based on their fusion with endosomes and lysosomes, resulting in their acidification and the acquisition of lytic enzymes (Vieira et al. 2002). Collectively, these processes result in the degradation of the internalized pathogen. After macrophages internalize and degrade bacteria, they present antigenic peptides on their major histocompatibility complex II (MHCII) and present it to T helper cells, which in turn activate B-cells to produce antigen-targeting antibodies (Hsieh et al. 1993a, b; Kahlert et al. 2000; Unanue and Askonas 1968; Hoffman et al. 2016).

4 Actin-Rich Uptake Structures: Filopodia and Coiling Pseudopods

To initiate phagocytosis, macrophages need to establish close physical contact with pathogens. Until recently, contact formation between an immune cell and its phagocytic target was seen as a more passive event, constituting a direct consequence of chemotactic immune cell migration and being followed by surface receptor clustering (Michl et al. 1983).

However, more recent work demonstrated that immune cells also actively enhance the probability of securing a target at their surface, by probing their environment with filopodia, receptor-containing cellular protrusions (Flannagan et al. 2010). Filopodia are elongated, finger-like protrusions of cells that contain bundles of linear actin filaments and can extend from the cell surface up to several tens of microns (Svitkina et al. 2003; Mallavarapu and Mitchison 1999). They are highly dynamic and constantly extend and retract, which is based on actin filament dynamics. Considering that macrophages mostly migrate in a three-dimensional environment and are embedded in the network of the extracellular matrix, an array of actively probing filopodial protrusions allows these cells to scan a much larger volume of space, compared to their actual cell body.

Filopodia dynamics are tightly regulated. Accordingly, receptor–ligand interactions established upon capturing a target are believed to induce a signaling cascade that leads to reduction of filopodia elongation, and instead favoring their retraction (Romero et al. 2011). However, clear evidence for the existence of such a signaling cascade is missing, and potentially involved molecular regulators remain to be identified.

Importantly, filopodia are also able to exert forces in the range of several hundreds of piconewtons (pN). This allows them to pull on attached particles, thereby bringing them into close contact with the surface of the host cell (Heidemann et al. 1990; Vonna et al. 2007; Kress et al. 2007). It was demonstrated that filopodia are able to pull persistently on objects during their retraction. Accordingly, filopodia of RAW (Abelson leukemia virus-transformed murine macrophage-like) cells that pulled on IgG-coated beads (Kress et al. 2007) or sheep red blood cells (Flannagan et al. 2010) were able to resist applied counterforce by an optical trap. Interestingly, the retraction speed of the bead-pulling filopodia slowed down in relation to the force applied by the optical trap (Kress et al. 2007). In contrast, macrophages that were treated with the F-actin stabilizing agent jasplakinolide, thus inhibiting actin turnover (Cramer 1999), failed to maintain this interaction (Flannagan et al. 2010). This latter experiment emphasizes the essential role of actin cytoskeleton dynamics for filopodia-mediated capturing of phagocytic targets.

The ability to maintain the attachment to objects despite applied counterforce becomes especially important in situations when phagocytes have to capture highly motile pathogens at their surface and prevent them from detaching. *B. burgdorferi*, equipped with periplasmic flagella, is an excellent example for such a highly motile pathogen. Intra-vital imaging showed the spirochetes are able to move at a speed of

4 μm/sec in murine ear tissue (Moriarty et al. 2008), similar to the speed of 4.25 μm/sec measured in vitro (Goldstein et al. 1994).

Intriguingly, coincubation of primary human macrophages with borreliae strongly enhanced filopodia formation per cell as compared to control cells (3.0 ± 0.2 in borrelia stimulated cells compared to 1.2 ± 0.2 in control cells) (Naj et al. 2013) (Fig. 2). Also, *Borrelia*-induced filopodia were longer compared to those of unstimulated cells [6.2 μm ± 0.4 μm for filopodia in borreliae stimulated macrophages, 3.2 μm ± 0.3 μm for unstimulated macrophages (Naj et al. 2013)]. Moreover, quantification of filopodia formation in cells stimulated solely with *Borrelia* culture supernatant revealed no difference compared to unstimulated cells, supporting the hypothesis that induction of filopodia is based on direct interaction of macrophages with pathogens, and not on soluble factors (Hoffmann et al. 2014; Naj et al. 2013). However, the respective molecular mechanism responsible for *Borrelia*-induced upregulation of filopodia formation is currently unclear.

Filopodia-dependent capturing by host cells has also been demonstrated for other bacteria such as enteroinvasive *Shigella flexneri* (Romero et al. 2011). In this case, entry of *Shigella* triggers opening of connexin-dependent hemichannels, resulting in enhanced levels of extracellular ATP, which in turn increased filopodia-mediated attachment of *Shigella* to Hela cells. This was accompanied by enhanced Erk1/2 activity, which was shown to be important for efficient filopodia retraction. It is an intriguing speculation that similar events might also play a role in *Borrelia* capturing by macrophage filopodia.

Comparable to the described experiments using latex beads, motile borreliae that were contacted by macrophage filopodia often remained in direct contact with the protrusions, reinforcing the notion that filopodia indeed constitute cellular organelles for capturing of pathogens, which are able to adhere continuously to a contacted bacterium. Moreover, multiple filopodia were often observed to surround captured borreliae at the cell surface of macrophages (Fig. 3), which might also point to a role for filopodia as a physical barrier that further hinders the escape of surface-attached spirochetes.

After borreliae are captured and brought close to the cell surface, they are preferentially internalized by a specific mechanism called coiling phagocytosis (Rittig et al. 1992) During this process, spirochetes are progressively enwrapped by a long actin-rich cell protrusion that arises from the cell surface at the *Borrelia*-contact site, surrounding the spirochete in multiple whorls (Fig. 1). Experiments using live or heat-inactivated borreliae (Rittig et al. 1998b; Rechnitzer and Blom 1989) and also other spirochetes showed that coiling phagocytosis is a host cell-driven process, which is probably based on the specific morphology of spirochetes and does not depend on the viability of bacteria.

Indeed, the phenomenon of coiling phagocytosis has been known for decades and was described for the uptake of several pathogens, including *Legionella pneumophila* (Horwitz 1984), *Trypanosoma cruzi*, *Leishmania spp*. as well as several fungal cells (Rittig et al. 1998c). It was found that coiling pseudopods are induced to the same extent by live as by killed pathogens (Rittig et al. 1998b; Rechnitzer and Blom 1989). Furthermore, neither supernatant of bacteria culture

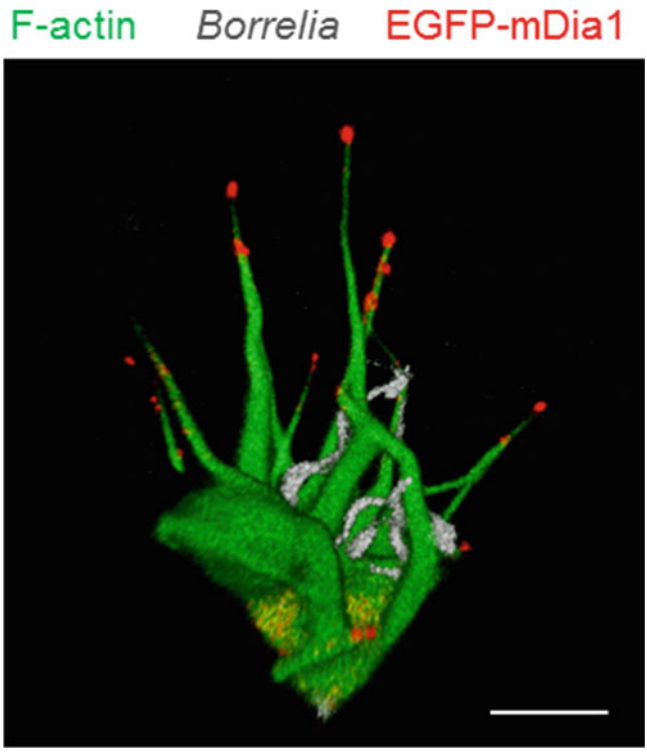

Fig. 3 Macrophage filopodia facilitate contact between borreliae and the host cell. 3D reconstruction using confocal Z-stacks of a macrophage stained for F-actin using Alexa 568 phalloidin (*green*) and expressing EGFP-mDia1 (*red*), with DNA of borreliae stained by Hoechst 33258 (*gray*). 3D reconstruction shows part of the macrophage surface that is in contact with several spirochetes, which are encaged by actin-rich filopodia. Note punctate enrichment of EGFP-mDia1 at the tips of filopodia. Scale bar: 5 μm

nor by sonification fragmented bacteria triggered pseudopod formation (Rittig et al. 1998b). Moreover, it has not been possible to relate this mechanism to any specific type of phagocytic receptor (Rittig et al. 1992), though coiling phagocytosis of borreliae shows characteristics of both CR3- and Fcγ-dependent phagocytosis (Linder et al. 2001). This again suggests that the helix-like structure of the coiling pseudopod is most probably a result of the spirochete morphology rather than being based on a particular ligand–receptor interaction (Rittig et al. 1998a, c).

This coiling pseudopod is highly flexible and contains multiple bending nodes, and is thus clearly distinct from the rather stiff filopodia (Figs. 1, 2, 3 and 4). Its flexible morphology allows the pseudopod to closely align along the spirally shaped body of the *Borrelia* cell (Naj et al. 2013; Hoffmann et al. 2014). Individual observations by live-cell imaging have shown that full internalization can require longer than 40 min (Naj and Linder 2015).

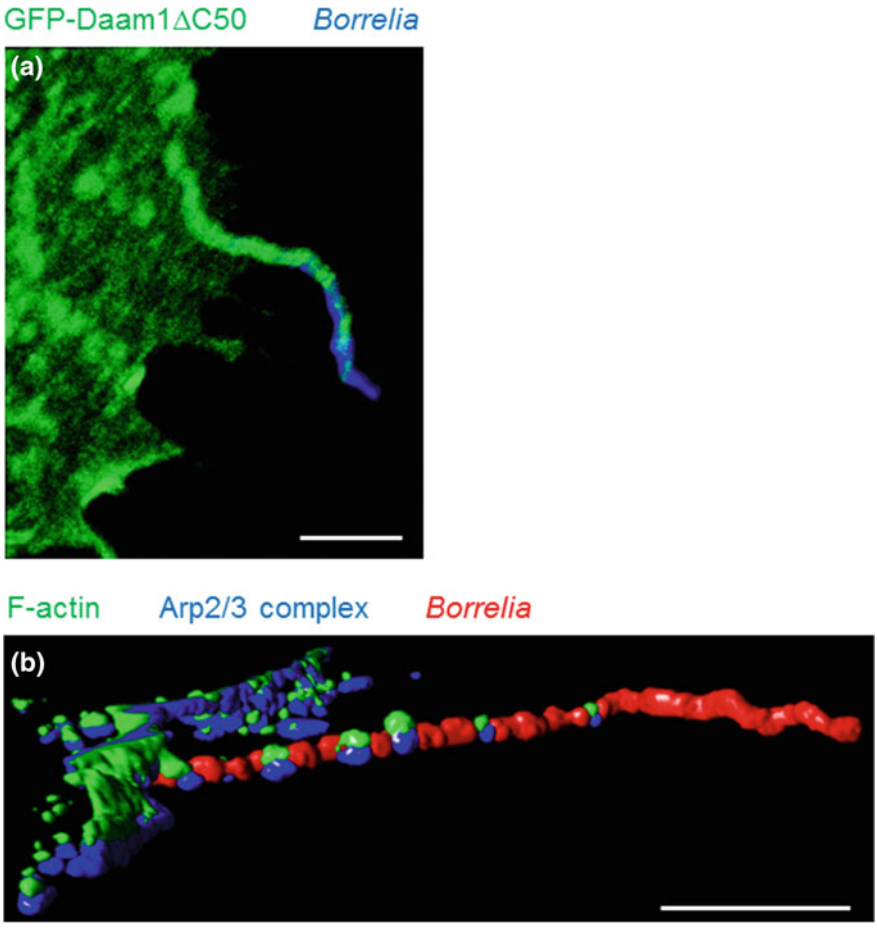

Fig. 4 Macrophage coiling pseudopods enwrapping borreliae are enriched in actin regulators. (**a**) Enrichment of GFP-Daam1ΔC50 during phagocytosis of borreliae. Confocal micrographs of primary human macrophage expressing GFP-Daam1ΔC50, a non-autoinactivated mutant (*green*) which is accumulated at the uptake structure of a *Borrelia* cell visualized by Hoechst 33342 staining of DNA (*blue*). (b) Isosurface reconstruction of confocal Z-stack, using Volocity software. *Borrelia* cell stained using antibody specific for OspA surface antigen (*red*), macrophage protrusion stained for F-actin (*green*) and Arp2/3 complex (*blue*). Macrophage cell body not shown. Note dot-like enrichment of Arp2/3 complex along the *Borrelia* cell, typical for coiling phagocytosis. Scale bars: 5 μm

Considering the more recently discovered involvement of actin-based filopodia prior to the formation of the coiling pseudopod during *Borrelia* phagocytosis, it was unclear whether both structures are formed independently or whether the coiling pseudopod arises from enhanced lateral growth of the already existing filopodia. Importantly, live-cell imaging experiments showed that coiling pseudopods indeed constitute separate structures that are formed de novo after borreliae are captured by

filopodia, and are not developed by further growth of filopodia that are already in contact with spirochetes This is also reflected by the distinct requirement for different actin regulators during the formation of either structure.

5 Actin Dynamics During *Borrelia* Phagocytosis: The Roles of Formins and Arp2/3 Complex

Formation and restructuring of actin filaments in cells is exquisitely controlled on many levels, to ensure exact formation of the required structures in time and space. Filaments can be formed de novo by nucleation or through fragmentation of existing ones. Elongation of filaments can be promoted or stopped by respective regulators, and dissolution is driven by processive disassembly or by severing into smaller filaments. Finally, individual filaments can be associated into higher ordered structures by bundling or crosslinking factors (Mellor 2010; Mattila and Lappalainen 2008).

Formation of filopodia involves the nucleation and elongation of unbranched actin filaments, as well as their connection in higher ordered bundles, to achieve the required stiffness. Of note, the formins FMNL1 and mDia1 have been localized to borreliae-induced filopodia, and their activity was shown to be required for filopodia formation in response to macrophage contact with borreliae, and also for subsequent internalization (Naj et al. 2013). Proteins of the formin family, 15 of which are expressed in human tissues (Schonichen and Geyer 2010), are important regulators of unbranched filaments. In principle, they are able to regulate all of the actin-related activities described above, including nucleation, elongation, capping, depolymerization, severing, and bundling with the individual set of abilities varying widely between the different isoforms (Schonichen and Geyer 2010; Grikscheit and Grosse 2016; Bohnert et al. 2013). In vitro, FMNL1 displays actin severing activity, thus giving rise to free barbed ends that can be used for the growth of new actin filaments (Harris et al. 2004), while mDia1 shows actin elongation and crosslinking activity (Li and Higgs 2003; Esue et al. 2008).

Furthermore, FMNL1 was localized along the whole shaft of borreliae-induced filopodia, whereas mDia1 showed a dot-like accumulation at the tips of filopdia (Naj et al. 2013) (Fig. 3). Combining both sets of observations, it is thus likely that mDia1 at the tips of filopodia regulates the growth of these structures by elongation of actin filaments, while FMNL1 might be involved in filopodia growth through the generation of free barbed ends along the shaft of the structure (Fig. 5). Structural stability of filopodia is supplied by bundling of actin filaments through fascin, which, comparable to FMNL1, localizes at the filopodial shaft (Naj et al. 2013). Of note, filament bundling is probably further supported through the activity of yet another formin, Daam1 (Hoffmann et al. 2014). Like FMNL1, endogenous and overexpressed forms of Daam1 were found to localize along the whole shaft of borreliae-induced and fascin-positive filopodia (Fig. 5).

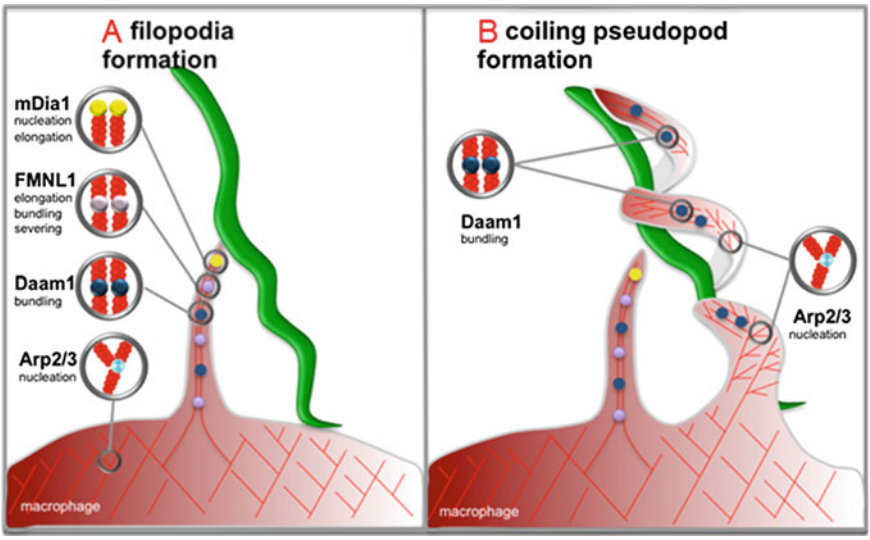

Fig. 5 Model of formin- and Arp2/3 complex dependent actin regulation in coiling phagocytosis of *Borrelia*. (**a**) Upon stimulation with borreliae, macrophages form filopodial protrusions that arise from the cortical network. Filopodia are enriched in the formins mDia1 (localized at tips) and FMNL1 (localized at tips and shaft), which probably contribute to longitudinal growth of filopodia, and Daam1 (localized in filopodial shaft), which is probably involved through its actin-bundling activity. (**b**) Upon capturing of a *Borrelia* cell by filopodia, the spirochete is enwrapped by a coiling pseudopod. Until recently, it was unclear whether coiling pseudopods develop from filopodia or constitute independent structures. Live-cell experiments showed that Daam1-positive coiling pseudopods arise as a second independent structure from the macrophage surface and enwrap borreliae. The flexibility of coiling pseudopods that enables them to enwrap the spiral-shaped borreliae is probably due to dot-like accumulations of Arp2/3 complex, which lead to formation of small branched actin networks and probably act as "hinges" at coiling nodes

This requirement for more than one bundling factor is surprising, but fascin has been shown to stabilize Daam1 at filopodia in B16F1 mouse melanoma cells, and silencing of Daam1 in these cells led to a decrease in the number of filopodia and also defects in their architecture (Jaiswal et al. 2013), pointing to a cooperative role of Daam1 and fascin in both formation and stabilization of filopodia. Similarly, siRNA-mediated knockdown of Daam1 in human macrophages resulted in a two-third reduction of filopodia formed upon contact with borreliae (Hoffmann et al. 2014), comparable to the effect of a fascin knockdown in these cells (Hoffmann et al. 2014). Interestingly, knockdown of Daam1 had a more pronounced effect on the number of filopodia than knockdown of either FMNL1 or mDia1 (40–50 % reduction each), which may point to the relative importance of Daam1 in filopodia formation or stabilization. Combined knockdown of all three formins, however, had no additive effect, showing that these formins work in the same pathway that ensures efficient filopodia formation (Hoffmann et al. 2014). Of note, the requirement for specific formins in filopodia regulation has been shown to

vary between cell types, and especially between adherent and suspension cells (Young et al. 2015). Thus, also the relative importance for FMNL1, mDia1, and Daam1 for formation of *Borrelia*-capturing filopodia may vary, depending on the type of immune cell involved, and also on the two- or three-dimensional context in which borreliae are encountered by immune cells in the body.

Inside–outside stainings of formin-depleted macrophages also showed that reduction of filopodia resulted in a ~50 % decrease of internalized borreliae, demonstrating that capturing of spirochetes by filopodia is an important step for efficient internalization by macrophages (Hoffmann et al. 2014). This is probably based on the effects that 1) filopodia allow cells to scan a larger volume of space and 2) immobilization of the highly motile spirochetes allows more time for the development of the coiling pseudopod, which is in most cases the decisive surface structure mediating phagocytosis of borreliae (Rittig et al. 1992; Naj et al. 2013).

Interestingly, live-cell imaging revealed that Daam1 apparently plays a dual role during uptake of borreliae by macrophages: not only through stabilization of borreliae-capturing filopodia, but also through formation of the coiling pseudopod itself (Hoffmann et al. 2014) (Fig. 4). This observation also resolved the question whether the coiling pseudopod forms independently from filopodia or through lateral growth of these structures. As the primary biochemical function of Daam1 is bundling of actin filaments, it is likely that this formin also works as an actin-bundling factor in the coiling pseudopod (Fig. 5).

This leaves the question how actin filaments in coiling pseudopods are nucleated or elongated. A partial answer to this lies in the localization of actin-nucleating Arp2/3 complex and its activator WASP (Wiskott-Aldrich Syndrome protein) at coiling pseudopods of macrophages (Linder et al. 2001). Interestingly, Arp2/3 complex has been shown to form dot-like accumulations along the coiling pseudopod, which often coincide with helical turns of the spirochete body (Fig. 4). It is thus tempting to speculate that Arp2/3 complex, as a generator of branched actin filament networks (Amann and Pollard 2001) provides nodes of branched actin that may alternate with sections of unbranched actin filaments, thus bringing the necessary flexibility to the coiling pseudopod structure that has to closely follow the helical spirochete morphology (Fig. 5). Of note, Arp2/3 complex has also been shown to be important for a subset of filopodia, by providing localized actin networks as a structural basis for their longitudinal extension (Young et al. 2015). However, the potential impact of Arp2/3 complex on borreliae-induced filopodia has not been tested yet.

An important upstream activator of WASP is Cdc42, a small GTPase of the Rho family (Mullins 2000). Accordingly, microinjection of dominant negative Cdc42 strongly reduced coiling pseudopod formation (Linder et al. 2001). It is thus very likely that an activation cascade Cdc42-WASP-Arp2/3 complex regulates formation of borreliae-induced coiling pseudopods. Furthermore, Cdc42 and other RhoGTPases, most notably RhoA or Rac1, may also be involved in the regulation of formin-dependent activities during *Borrelia* capturing and internalization. Formins are usually autoinhibited by backfolding of an inhibitory DID domain (diaphanous inhibitory domain) to a regulatory DAD domain (diaphanous

autoregulatory domain), and only binding of RhoGTPases and other factors leads to release of this autoinhibition and to full activity of formins (Kuhn and Geyer 2014; Higgs 2005). Accordingly, many of the experiments regarding the involvement of formins in borreliae-induced filopodia formation were performed using non-autoinhibited constructs such as Daam1ΔC50 that lack the respective DID domains and thus circumvent the need for RhoGTPase-dependent activation. An important role for RhoGTPases in borreliae-capturing filopodia is thus highly likely, although the involvement of specific RhoGTPases in activation of respective formins has not been tested yet.

Collectively, these data lead to the following multistep model of *Borrelia* phagocytosis by macrophages: (1) physical contact of borreliae with macrophages leads to increased formation of filopodia that are able to contact and bind borreliae, thus leading to immobilization of the highly motile spirochets on the bacterial surface. Filopodia formation depends on the concerted activity of three formins, FMNL1, mDia1, and Daam1, which respectively regulate actin filament formation, elongation, and bundling, with further bundling activity provided by fascin. In a second phase, a filopodia-independent structure, the coiling pseudopod, arises from the macrophage surface. It closely follows the helical spirochete morphology and thus tightly enwraps captured borreliae (Fig. 5). Actin within coiling pseudopods is probably present alternatingly as elongated unbranched filaments that are bundled by Daam1 and as nodes of branched actin networks formed by Cdc42-WASP-Arp2/3-dependent actin nucleation. This architecture would allow the necessary flexibility that is required for this structure that enwraps the helical spirochete. Finally, the coiling pseudopod has to contract, to be brought in close contact with the macrophage surface, and the captured borreliae have to be internalized. These steps likely involve regulators of actin-based contractility such as myosin II and also disassembly of actin filaments, possibly necessitating further formin activity. This should prove to be a fertile field for future research. Of note, due to their elongated morphology, intracellular processing of captured borreliae can already be in progress, as outlined below, even when extracellular parts of the spirochetes are still being enwrapped by actin-driven coiling pseudopods.

6 Intracellular Processing of *Borrelia*—A Central Role for RabGTPases

During phagocytosis, bacteria enter the cell in a membrane-delimited compartment termed the phagosome. Apart from the internalized target, the phagosome is initially filled with fluids that derive from the extracellular space. Subsequent alteration in phagosome composition proceeds through highly coordinated exchange of material with vesicles of the endomembrane system (Fairn and Grinstein 2012). Ultimately, these steps lead to the maturation of the phagosome into an acidic, oxidative compartment that is enriched in hydrolytic enzymes. According to the

enrichment and/or loss of respective marker proteins and also to the progressive drop of the intraluminal pH, phagosomes are classified into distinct stages: (i) early phagosome, (ii) late phagosome, and (iii) phagolysosome. The fully matured phagolysosome is able to digest lipids, proteins, and carbohydrates and thus neutralizes infecting bacteria.

Key regulators of this maturation process include members of the Rab (Ras-related proteins in brain) GTPase family, which are molecular switches cycling between their active GTP-bound and inactive GDP-bound state (Vieira et al. 2002; Stenmark et al. 1994). In their active state, RabGTPases interact with their respective effector proteins that control various processes including phagosomal membrane fusion and fission events and motor-dependent transport (Gautreau et al. 2014; Hutagalung and Novick 2011).

In this respect, it could be shown that borreliae are initially internalized into a Rab22a positive phagosome. Subsequently, the phagosome is contacted by Rab5a positive vesicles. Quickly after internalization, the elongated spirochetes are compacted into globular, dense structures. This striking compaction of borreliae is accompanied by repeated tubulation of membrane from phagosomes, suggesting that reduction of the phagosomal surface could be a driving force for spirochete compaction. Interestingly, fission of membrane tubules occurs preferentially at sites where Rab5a positive vesicles contact the Rab22a positive phagosomal membrane, indicating that the concerted activity of both RabGTPases is necessary for this process (Naj and Linder 2015) (Fig. 6).

Previous reports showed that the endoplasmic reticulum (ER) contacts Rab5 positive early endosomes at subdomains just before fission occurs at those sites (Rowland et al. 2014). Consistent with the notion that similar processes could be involved in the membrane fission events from the phagosomes during *Borrelia* compaction, it was observed that the ER forms a network around *Borrelia*-containing phagosomes and that Rab5a vesicles contact the Rab22a-positive phagosomal membrane along the ER (Naj and Linder 2015) (Fig. 6).

Moreover, an F-actin coat was detected to surround the early, *Borrelia*-containing phagosome (Naj and Linder, unpublished). It is therefore conceivable that F-actin plays a role during *Borrelia*-compaction and the concomitant membrane tubule fission events during this process. Indeed, previous studies showed that WASH, a vesicle-localized Arp2/3 complex activator of the WASP family (Gautreau et al. 2014) regulates membrane fission from Rab5-positive early endosomes (Duleh and Welch 2010). It is thus tempting to speculate that WASH-mediated actin polymerization at phagosomes could contribute to the observed membrane fission events that occur during *Borrelia* compaction. However, this concept requires further evaluation.

Furthermore, Rab22a and Rab5a knockdown not only inhibited the compaction of the spirochetes, but also the maturation of *Borrelia*-containing phagosomes. This was demonstrated by decreased ($\sim$20–40 %) lysosomal associated membrane protein1 (LAMP1) acquisition at the phagosomal membrane (Naj and Linder 2015) and also by decreased ($\sim$15–45 %) phagosomal colocalization with DQ-BSA (Naj and Linder 2015), a marker for proteolytic activity (Fig. 6). Moreover, knockdown

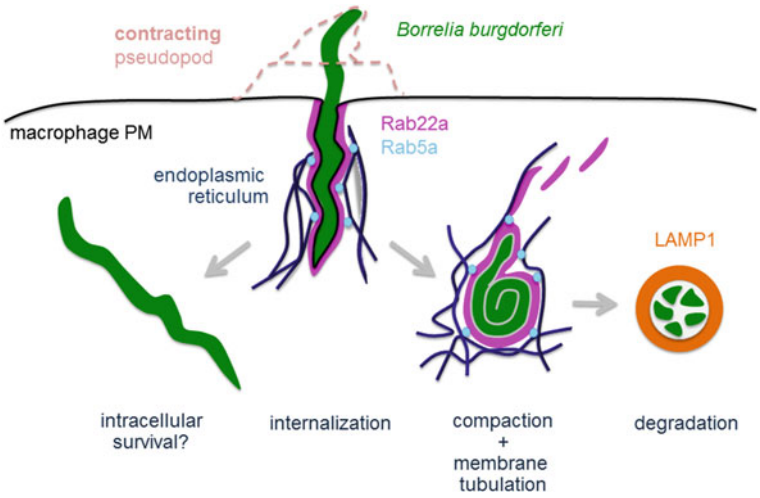

Fig. 6 Model of intracellular processing of borreliae by macrophages. Borreliae captured by macrophages via coiling pseudopods are internalized through uptake into Rab22a-positive phagosomes. Phagosomes are subsequently contacted by Rab5a positive vesicles mediated by the endoplasmic reticulum. Subsequent membrane tubulation causes reduction of the phagosome surface, leading to visible compaction of borreliae. Further maturation of this compartment leads to its development into a degradative phagolysosome, indicated by the presence of the lyosomal marker protein LAMP1, and resulting in elimination of spirochetes. In contrast, escape from Rab22a-/Rab5a-dependent processing can lead to enhanced intracellular survival of borreliae. Modified from Naj and Linder (2015), with permission

of Rab22a and Rab5a in macrophages led to enhanced (~6 fold) intracellular survival of the spirochetes (Naj and Linder 2015).

Of note, the specific subset of RabGTPases can vary between phagosomes that contain different bacteria. Moreover, several bacteria have evolved strategies to influence RabGTPase recruitment and/or activity as part of an escape strategy to influence phagosome maturation and thus avoid being degraded (Smith and May 2013). It is thus noteworthy that a small subpopulation of borreliae (~5 %) colocalized only transiently with Rab22a and Rab5a, did not undergo compaction and retained their elongated morphology (Naj and Linder 2015). Indeed, former electron microscopy studies demonstrated the presence of elongated borreliae localized within the cell cytoplasm without any clearly detectable phagosomal membrane (Filgueira et al. 1996; Ionescu et al. 1997; Hechemy et al. 1992). It is thus conceivable that this could be a subpopulation, which escapes the phagosome. Supporting this notion, heat killed borreliae were detected consistently surrounded by a phagosomal membrane in Vero cells (Hechemy et al. 1992). In contrast, borreliae are not known to harbor any secretion system or to express any virulence factors (Fraser et al. 1997). Closer investigation of this hypothetical subset of borreliae, their potential to persist in human immune cells, as well as the possible molecular mechanisms involved, should thus be an interesting challenge for the future.

7 Conclusions

Efficient uptake and elimination of borreliae by immune cells is crucial for countering the development of Lyme disease. In particular, macrophages form an important part of the initial defense line that prevents dissemination of *B. burgdorferi* within the host through a carefully orchestrated succession of capturing, internalization, and degradation of spirochetes.

In this context, recent research has highlighted the role of local restructuring of the macrophage actin cytoskeleton, which enables the formation of specific surface structures that interact with infecting spirochetes. First, macrophages respond to the presence of borreliae by forming filopodia, long, rigid protrusions that contain a core of linear, bundled actin filaments. Filopodia enable the capturing and immobilization of the highly motile spirochetes. Second, a filopodia-independent structure is formed, the coiling pseudopod, which enwraps captured borreliae and promotes their internalization. Closely following the helical morphology of the spirochete, this structure requires a more flexible arrangement of actin filaments.

Accordingly, these different structures have been shown to depend on different subsets of actin regulators, with *Borrelia*-induced filopodia depending the formins FMNL1 and mDia1, regulators of unbranched actin filaments, while the coiling pseudopod apparently contains nodes of Arp2/3 complex-generated branched actin networks. In addition, both structures depend on the activity of the formin Daam1.

Due to the elongated morphology of the *Borrelia* cell, intracellular processing of internalized parts of the spirochete can happen concomitantly with the uptake of still extracellular parts of the spirochete. Internalized borreliae have been shown to enter phagosomes, which is accompanied by successive compaction of borreliae. Further maturation of phagosomes into degradative lysosomes involves the RabGTPases Rab22a and Rab5a. The activities of both RabGTPases are coordinated by the endoplasmic reticulum, which closely enwraps the internalized parts of the spirochete, thus forming an intracellular counterpart to the coiling pseudopod that enwraps the extracellular parts of the spirochete.

Collectively, these novel insights into the subcellular and molecular regulation of *Borrelia* capturing, uptake, and degradation illustrate the highly efficient mechanisms that macrophages have developed to counter respective infections. On the other hand, it will be highly interesting to determine if and to which extent *B. burgdorferi* is able to counter these mechanisms and to thus support its dissemination in the human host.

Finally, the unique spirochete morphology of *Borrelia* has enabled the detection of subcellular mechanisms during uptake and processing by macrophages that would be difficult to visualize using more globular bacteria. *B. burgdorferi* is thus also emerging as a useful tool for the detailed study of organelle interactions during phagocytosis in general.

Acknowledgments We apologize to all whose work was not mentioned owing to space limitations. We thank Mirko Himmel for help with figures, Andrea Mordhorst for expert technical assistance and Martin Aepfelbacher for continuous support. Work from the SL lab mentioned in this article has been supported by the Deutsche Forschungsgemeinschaft (SPP1464, GRK 1459).

References

Aberer E, Duray PH (1991) Morphology Of Borrelia burgdorferi: structural patterns of cultured Borreliae in relation to staining methods. J Clin Microbiol 29:764–772

Aguero-Rosenfeld ME, Wormser GP (2015) Lyme disease: diagnostic issues and controversies. Expert Rev Mol Diagn 15:1–4

Amann KJ, Pollard TD (2001) The Arp2/3 complex nucleates actin filament branches from the sides of pre-existing filaments. Nat Cell Biol 3:306–310

Benach JL, Fleit HB, Habicht GS, Coleman JL, Bosler EM, Lane BP (1984) Interactions of phagocytes with the lyme disease spirochete: role of the Fc receptor. J Infect Dis 150:497–507

Berende A, Oosting M, Kullberg BJ, Netea MG, Joosten LA (2010) Activation of innate host defense mechanisms by Borrelia. Eur Cytokine Netw 21:7–18

Berndtson K (2013) Review of evidence for immune evasion and persistent infection in lyme disease. Int J Gen Med 6:291–306

Bohnert KA, Willet AH, Kovar DR, Gould KL (2013) Formin-based control of the actin cytoskeleton during cytokinesis. Biochem Soc Trans 41:1750–1754

Borgermans L, Goderis G, Vandevoorde J, Devroey D (2014) Relevance of chronic lyme disease to family medicine as a complex multidimensional chronic disease construct: a systematic review. Int J Family Med 2014:138016

Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP (1982) Lyme disease-A tick-borne spirochetosis? Science 216:1317–1319

Carrasco SE, Troxell B, Yang Y, Brandt SL, Li H, Sandusky GE, Condon KW, Serezani CH, Yang XF (2015) Outer surface protein Ospc is an antiphagocytic factor that protects Borrelia burgdorferi from phagocytosis by macrophages. Infect Immun 83:4848–4860

Cinco M, Murgia R, Presani G, Perticarari S (1997) Integrin Cr3 mediates the binding of nonspecifically opsonized Borrelia burgdorferi to human phagocytes and mammalian cells. Infect Immun 65:4784–4789

Cramer LP (1999) Role of actin-filament disassembly in lamellipodium protrusion in motile cells revealed using the drug jasplakinolide. Curr Biol 9:1095–1105

Duleh SN, Welch MD (2010) Wash and the Arp2/3 complex regulate endosome shape and trafficking. Cytoskeleton (Hoboken) 67:193–206

Duray PH (1989) Histopathology of clinical phases of human lyme disease. Rheum Dis Clin North Am 15:691–710

Embers ME, Barthold SW, Borda JT, Bowers L, Doyle L, Hodzic E, Jacobs MB, Hasenkampf NR, Martin DS, Narasimhan S, Phillippi-Falkenstein KM, Purcell JE, Ratterree MS, Philipp MT (2012) Persistence of Borrelia burgdorferi in rhesus macaques following antibiotic treatment of disseminated infection. PLoS ONE 7:E29914

Esue O, Harris ES, Higgs HN, Wirtz D (2008) The filamentous actin cross-linking/bundling activity of mammalian formins. J Mol Biol 384:324–334

Fairn GD, Grinstein S (2012) How nascent phagosomes mature to become phagolysosomes. Trends Immunol 33:397–405

Filgueira L, Nestle FO, Rittig M, Joller HI, Groscurth P (1996) Human dendritic cells phagocytose and process Borrelia burgdorferi. J Immunol 157:2998–3005

Flannagan RS, Harrison RE, Yip CM, Jaqaman K, Grinstein S (2010) Dynamic macrophage "Probing" is required for the efficient capture of phagocytic targets. J Cell Biol 191:1205–1218

Fraser CM, Casjens S, Huang WM, Sutton GG, Clayton R, Lathigra R, White O, Ketchum KA, Dodson R, Hickey EK, Gwinn M, Dougherty B, Tomb JF, Fleischmann RD, Richardson D, Peterson J, Kerlavage AR, Quackenbush J, Salzberg S, Hanson M, Van Vugt R, Palmer N, Adams MD, Gocayne J, Weidman J, Utterback T, Watthey L, Mcdonald L, Artiach P, Bowman C, Garland S, Fuji C, Cotton MD, Horst K, Roberts K, Hatch B, Smith HO, Venter JC (1997) Genomic sequence of a lyme disease spirochaete, Borrelia burgdorferi. Nature 390:580–586

Garcia RC, Murgia R, Cinco M (2005) Complement receptor 3 binds the Borrelia burgdorferi outer surface proteins Ospa and Ospb in an Ic3b-independent manner. Infect Immun 73:6138–6142

Gautreau A, Oguievetskaia K, Ungermann C (2014) Function and regulation of the endosomal fusion and fission machineries. Cold Spring Harb Perspect Biol 6

Goldstein SF, Charon NW, Kreiling JA (1994) Borrelia burgdorferi swims with a planar waveform similar to that of eukaryotic flagella. Proc Natl Acad Sci USA 91:3433–3437

Grikscheit K, Grosse R (2016) Formins at the junction. Trends Biochem Sci 41:148–159

Harris ES, Li F, Higgs HN (2004) The mouse formin, frlalpha, slows actin filament barbed end elongation, competes with capping protein, accelerates polymerization from monomers, and severs filaments. J Biol Chem 279:20076–20087

Hawley KL, Olson CM Jr, Iglesias-Pedraz JM, Navasa N, Cervantes JL, Caimano MJ, Izadi H, Ingalls RR, Pal U, Salazar JC, Radolf JD, Anguita J (2012) Cd14 cooperates with complement receptor 3 to mediate Myd88-independent phagocytosis of Borrelia burgdorferi. Proc Natl Acad Sci U S A 109:1228–1232

Hechemy KE, Samsonoff WA, Harris HL, Mckee M (1992) Adherence and entry of Borrelia burgdorferi in vero cells. J Med Microbiol 36:229–238

Heidemann SR, Lamoureux P, Buxbaum RE (1990) Growth cone behavior and production of traction force. J Cell Biol 111:1949–1957

Higgs HN (2005) Formin proteins: a domain-based approach. Trends Biochem Sci 30:342–353

Hoffmann AK, Naj X, Linder S (2014) Daam1 is a regulator of filopodia formation and phagocytic uptake of Borrelia burgdorferi by primary human macrophages. Faseb J 28:3075–3089

Hoffman W, Lakkis FG, Chalasani G (2016) B cells, antibodies, and more. Clin J Am Soc Nephrol 11:137–154

Horwitz MA (1984) Phagocytosis of the legionnaires' disease bacterium (Legionella pneumophila) occurs by a novel mechanism: engulfment within a pseudopod coil. Cell 36:27–33

Hsieh CS, Macatonia SE, O'garra A, Murphy KM (1993a) Pathogen-induced Th1 phenotype development in Cd4+ alpha beta-Tcr transgenic T cells is macrophage dependent. Int Immunol 5:371–382

Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'garra A, Murphy KM (1993b) Development of Th1 Cd4+ T cells through Il-12 produced by listeria-induced macrophages. Science 260:547–549

Hutagalung AH, Novick PJ (2011) Role of rab Gtpases in membrane traffic and cell physiology. Physiol Rev 91:119–149

Ionescu MD, Ionescu AD, Hristescu S, Ionescu M, Orasanu M, Coman N (1997) Interactions between Borrelia burgdorferi and eukaryote cells: comparative ultrastructural aspects. Roum Arch Microbiol Immunol 56:77–96

Jaiswal R, Breitsprecher D, Collins A, Correa IR Jr, Xu MQ, Goode BL (2013) The formin daam1 and fascin directly collaborate to promote filopodia formation. Curr Biol 23:1373–1379

Jares TM, Mathiason MA, Kowalski TJ (2014) Functional outcomes in patients with Borrelia burgdorferi reinfection. Ticks Tick Borne Dis 5:58–62

Kahlert H, Grage-Griebenow E, Stuwe HT, Cromwell O, Fiebig H (2000) T cell reactivity with allergoids: influence of the type of Apc. J Immunol 165:1807–1815

Kress H, Stelzer EH, Holzer D, Buss F, Griffiths G, Rohrbach A (2007) Filopodia act as phagocytic tentacles and pull with discrete steps and a load-dependent velocity. Proc Natl Acad Sci USA 104:11633–11638

Kuhn S, Geyer M (2014) Formins as effector proteins of Rho Gtpases. Small Gtpases 5:E29513

Kumar D, Ristow LC, Shi M, Mukherjee P, Caine JA, Lee WY, Kubes P, Coburn J, Chaconas G (2015) Intravital imaging of vascular transmigration by the lyme spirochete: requirement for the integrin binding residues of the B. burgdorferi P66 protein. PLoS Pathog 11:E1005333

Lane RS, Piesman J, Burgdorfer W (1991) Lyme borreliosis: relation of its causative agent to its vectors and hosts in North America and Europe. Annu Rev Entomol 36:587–609

Lawrenz MB, Wooten RM, Zachary JF, Drouin SM, Weis JJ, Wetsel RA, Norris SJ (2003) Effect of complement component C3 deficiency on experimental Lyme borreliosis in mice. Infect Immun 71:4432–4440

Li F, Higgs HN (2003) The mouse formin mdia1 is a potent actin nucleation factor regulated by autoinhibition. Curr Biol 13:1335–1340

Li C, Motaleb A, Sal M, Goldstein SF, Charon NW (2000) Spirochete periplasmic flagella and motility. J Mol Microbiol Biotechnol 2:345–354

Linder S, Heimerl C, Fingerle V, Aepfelbacher M, Wilske B (2001) Coiling phagocytosis of Borrelia burgdorferi by primary human macrophages is controlled by Cdc42hs and Rac1 and involves recruitment of Wiskott-Aldrich syndrome protein and Arp2/3 complex. Infect Immun 69:1739–1746

Liu N, Montgomery RR, Barthold SW, Bockenstedt LK (2004) Myeloid differentiation antigen 88 deficiency impairs pathogen clearance but does not alter inflammation in Borrelia burgdorferi-infected mice. Infect Immun 72:3195–3203

Mallavarapu A, Mitchison T (1999) Regulated actin cytoskeleton assembly at filopodium tips controls their extension and retraction. J Cell Biol 146:1097–1106

Margos G, Vollmer SA, Ogden NH, Fish D (2011) Population genetics, taxonomy, phylogeny and evolution of Borrelia burgdorferi sensu lato. Infect Genet Evol 11:1545–1563

Mattila PK, Lappalainen P (2008) Filopodia: molecular architecture and cellular functions. Nat Rev Mol Cell Biol 9:446–454

Mellor H (2010) The role of formins in filopodia formation. Biochim Biophys Acta 1803:191–200

Michl J, Pieczonka MM, Unkeless JC, Bell GI, Silverstein SC (1983) Fc receptor modulation in mononuclear phagocytes maintained on immobilized immune complexes occurs by diffusion of the receptor molecule. J Exp Med 157:2121–2139

Montgomery RR, Nathanson MH, Malawista SE (1994) Fc- and Non-Fc-mediated phagocytosis of Borrelia burgdorferi by macrophages. J Infect Dis 170:890–893

Moriarty TJ, Norman MU, Colarusso P, Bankhead T, Kubes P, Chaconas G (2008) Real-time high resolution 3D imaging of the Lyme Disease spirochete adhering to and escaping from the vasculature of a living host. PLoS Pathog 4:E1000090

Mullins RD (2000) How Wasp-family proteins and the Arp2/3 complex convert intracellular signals into cytoskeletal structures. Curr Opin Cell Biol 12:91–96

Naj X, Linder S (2015) Er-coordinated activities of Rab22a and Rab5a drive phagosomal compaction and intracellular processing of Borrelia burgdorferi by macrophages. Cell Rep 12:1816–1830

Naj X, Hoffmann AK, Himmel M, Linder S (2013) The formins Fmnl1 and Mdia1 regulate coiling phagocytosis of Borrelia burgdorferi by primary human macrophages. Infect Immun 81:1683–1695

Pearson S (2014) Recognising and understanding Lyme Disease. Nurs Stand 29:37–43

Petzke M, Schwartz I (2015) Borrelia burgdorferi pathogenesis and the immune response. Clin Lab Med 35:745–764

Rechnitzer C, Blom J (1989) Engulfment of the philadelphia strain of Legionella pneumophila within pseudopod coils in human phagocytes. Comparison with other Legionella strains and species. Apmis 97:105–114

Rittig MG, Krause A, Haupl T, Schaible UE, Modolell M, Kramer MD, Lutjen-Drecoll E, Simon MM, Burmester GR (1992) Coiling phagocytosis is the preferential phagocytic mechanism for Borrelia burgdorferi. Infect Immun 60:4205–4212

Rittig MG, Burmester GR, Krause A (1998a) Coiling phagocytosis: when the zipper jams, the cup is deformed. Trends Microbiol 6:384–388

Rittig MG, Jagoda JC, Wilske B, Murgia R, Cinco M, Repp R, Burmester GR, Krause A (1998b) Coiling phagocytosis discriminates between different spirochetes and is enhanced by Phorbol Myristate acetate and granulocyte-macrophage colony-stimulating factor. Infect Immun 66:627–635

Rittig MG, Schroppel K, Seack KH, Sander U, N'diaye EN, Maridonneau-Parini I, Solbach W, Bogdan C (1998c) Coiling phagocytosis of trypanosomatids and fungal cells. Infect Immun 66:4331–4339

Romero S, Grompone G, Carayol N, Mounier J, Guadagnini S, Prevost MC, Sansonetti PJ, Van Nhieu GT (2011) Atp-mediated Erk1/2 activation stimulates bacterial capture by filopodia, which precedes shigella invasion of epithelial cells. Cell Host Microbe 9:508–519

Rowland AA, Chitwood PJ, Phillips MJ, Voeltz GK (2014) Er contact sites define the position and timing of endosome fission. Cell 159:1027–1041

Salazar JC, Pope CD, Sellati TJ, Feder HM Jr, Kiely TG, Dardick KR, Buckman RL, Moore MW, Caimano MJ, Pope JG, Krause PJ, Radolf JD (2003) Coevolution of markers of innate and adaptive immunity in skin and peripheral blood of patients with erythema migrans. J Immunol 171:2660–2670

Salazar JC, Duhnam-Ems S, La Vake C, Cruz AR, Moore MW, Caimano MJ, Velez-Climent L, Shupe J, Krueger W, Radolf JD (2009) Activation of human monocytes by live Borrelia burgdorferi generates Tlr2-dependent and -independent responses which include induction of Ifn-Beta. PLoS Pathog 5:E1000444

Schonichen A, Geyer M (2010) Fifteen formins for an actin filament: a molecular view on the regulation of human formins. Biochim Biophys Acta 1803:152–163

Shin OS, Miller LS, Modlin RL, Akira S, Uematsu S, Hu LT (2009) Downstream signals for Myd88-mediated phagocytosis of Borrelia burgdorferi can be initiated by Trif and are dependent on Pi3k. J Immunol 183:491–498

Smith LM, May RC (2013) Mechanisms of microbial escape from phagocyte killing. Biochem Soc Trans 41:475–490

Steere AC, Malawista SE, Snydman DR, Shope RE, Andiman WA, Ross MR, Steele FM (1977) Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three connecticut communities. Arthritis Rheum 20:7–17

Steere AC, Bartenhagen NH, Craft JE, Hutchinson GJ, Newman JH, Rahn DW, Sigal LH, Spieler PN, Stenn KS, Malawista SE (1983) The early clinical manifestations of Lyme Disease. Ann Intern Med 99:76–82

Steere AC, Coburn J, Glickstein L (2004) The emergence of Lyme Disease. J Clin Invest 113:1093–1101

Stenmark H, Parton RG, Steele-Mortimer O, Lutcke A, Gruenberg J, Zerial M (1994) Inhibition of Rab5 Gtpase activity stimulates membrane fusion in endocytosis. EMBO J 13:1287–1296

Stricker RB, Johnson L (2013) Borrelia burgdorferi aggrecanase activity: more evidence for persistent infection in Lyme Disease. Front Cell Infect Microbiol 3:40

Svitkina TM, Bulanova EA, Chaga OY, Vignjevic DM, Kojima S, Vasiliev JM, Borisy GG (2003) Mechanism of filopodia initiation by reorganization of a dendritic network. J Cell Biol 160:409–421

Unanue ER, Askonas BA (1968) The immune response of mice to antigen in macrophages. Immunology 15:287–296

Vieira OV, Botelho RJ, Grinstein S (2002) Phagosome maturation: aging gracefully. Biochem J 366:689–704

Vonna L, Wiedemann A, Aepfelbacher M, Sackmann E (2007) Micromechanics of filopodia mediated capture of pathogens by macrophages. Eur Biophys J 36:145–151

Wang G, Ma Y, Buyuk A, Mcclain S, Weis JJ, Schwartz I (2004) Impaired host defense to infection and toll-like receptor 2-independent killing of Borrelia burgdorferi clinical isolates in Tlr2-deficient C3h/Hej mice. FEMS Microbiol Lett 231:219–225

Wormser GP, Dattwyler RJ, Shapiro ED, Halperin JJ, Steere AC, Klempner MS, Krause PJ, Bakken JS, Strle F, Stanek G, Bockenstedt L, Fish D, Dumler JS, Nadelman RB (2006) The clinical assessment, treatment, and prevention of Lyme Disease, human granulocytic

anaplasmosis, and babesiosis: clinical practice guidelines by the infectious diseases Society of America. Clin Infect Dis 43:1089–1134

Young LE, Heimsath EG, Higgs HN (2015) Cell type-dependent mechanisms for formin-mediated assembly of filopodia. Mol Biol Cell 26:4646–4659

Zajkowska J, Lewczuk P, Strle F, Stanek G (2012) Lyme borreliosis: from pathogenesis to diagnosis and treatment. Clin Dev Immunol 2012:231657

Ziuzia Iu R, Efimova NS, Vorob'eva NN, Klitsunova NV, Gosteva VV (1999) Clinical and morphological characteristics of migrating erythema in patients with ixodes tick-borne Lyme Disease. Med Parazitol (Mosk) 36–41