The Many Faces of Bacterium-Endothelium Interactions during Systemic Infections

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Abstract

A wide variety of pathogens reach the blood circulation during viral, parasitic, fungal or bacterial infections, causing clinically diverse pathologies. Such systemic infections are usually particularly severe and frequently life-threatening despite intensive care, in particular at the age of antibiotic resistance. Because of its position at the interface between the blood and the rest of the organism, the endothelium plays a central role during these infections. Through several examples of systemic infections, we propose to explore the diversity of interactions between pathogens and the endothelium. These examples reveal that bacterial pathogens target specific vascular beds and affect most aspects of endothelial cell biology ranging from cellular junction stability to endothelial cell proliferation or inflammation.

THE ENDOTHELIUM AS A SITE OF BACTERIAL INFECTIONS

The endothelium refers to the layer of endothelial cells (ECs) lining the inner surface of blood vessels that span the entire body and ensure the distribution of blood throughout the organism (1). It can be estimated that the human body contains the staggering number of 100 000 km of blood vessels, more than twice the earth's circumference (2). Therefore, a bacterium reaching the circulation is engaged in a maze of huge proportion. Moreover, a pathogen travelling throughout the circulation does not encounter a homogeneous environment, as an important feature of the vascular network is its diversity. Although endothelial cells are present in all vessels, the organization of the vessel wall – formed by three layers referred to as the *tunica intima*, *media* and *adventia* (from the vessel lumen outward) – is different among different vessel types and different organs (3). Vessels can be first differentiated by the complex extracellular matrix layers surrounding them. For instance, elastic arteries such as the aorta are surrounded by 50 elastic layers providing them unique mechanical properties (3). Second, the cellular content is also different according to vessel type, the wall of arteries and veins contains a layer of smooth muscle cells (SMCs) that provides their capacity to relax or constrict in response to vasoactive molecules (4). An additional level of complexity in the network stems from the fact that larger vessels, veins or arteries are themselves vascularized by smaller vessels, the *vasa vasorum* (5). Although endothelial cells are constituent of all vessels, they themselves present different properties depending on their anatomical location, in particular in the case of capillaries. The lumen of *continuous capillaries,* which are the most common, are lined with an uninterrupted layer of endothelial cells. *Fenestrated capillaries*, typically present in glomeruli of the kidney, are laced with 50-80 nm openings thus changing their permeability properties. In the liver, *sinusoidal*

capillaries contain numerous holes that can reach several microns in diameter and could in principle allow objects such as bacteria to escape the circulation (6). Also, particularly relevant to infection, sinusoidal capillaries host a large number of Küpffer cells, phagocytic cells that constantly filter the blood from particulate matter including bacteria (7). Finally, the heart, a central element of the circulation network and also a potential site of infection, displays a specialized endothelium referred to as the *endocardium*. In contrast to the endothelium, the endocardium is constituted of three juxtaposed layers that ensure *i)* its physical anchorage to the surface of the myocardium (the heart muscle), *ii)* its mechano-elastic properties allowing its adaptation to the heart contraction and relaxation cycles and *iii)* its low permeability thanks to a sealed monolayer of endothelial cells (8).

Although usually viewed as a static structure, the design of the blood vessel network is dynamic, in particular in the case of the smaller vessels, first during development but also following wound healing, cancer development, ischemia or infection (9, 10). During development, *vasculogenesis* supports the establishment of the arteries and the veins that transport the blood from and back to the heart, respectively (11). An additional mechanism, referred to as *angiogenesis*, gives rise to smaller vessels, such as blood capillaries of few micrometers in diameter, which deliver oxygen and nutrients to the body's tissues. These vessels elongate from endothelial sprouts emanating from pre-existing vessels and invade non-vascularized areas (12). Of note, capillaries are also able to interconnect through anastomosis, a process resulting in the fusion of two capillary growing-ends (13). Therefore, pathogens reaching the circulation encounter a complex, diverse and dynamic network.

CELLULAR JUNCTIONS AS THE GATEKEEPERS OF THE ENDOTHELIAL BARRIER

Through the fine control of vessel permeability, intercellular junctions within the endothelium are at the heart of the maintenance of vascular integrity, thus ensuring the proper barrier function of the endothelium (14). Among the two main types of endothelial cell-cell junctions, *adherens* junctions (AJ) are ubiquitously found, whereas tight junctions (TJ) are mainly located in endothelial barriers with a very high selectivity (15). This is the case of the blood-brain barrier, where tight junctions ensure the charge- and size-selective exchanges between the cerebral vasculature and the central nervous system (CNS) (16), thus participating in the protection of the brain parenchyma from bacterial invasion, for instance. The main component of *adherens* junctions is the intercellular adhesion molecule Vascular Endothelial (VE)-Cadherin. VE-Cadherin proteins expressed at the surface of neighboring endothelial cells engage their extracellular domain within homotypic interactions that are stabilized by extracellular calcium (17), thus ensuring the sealing of the endothelium. Platelet-Endothelial Cell Adhesion Molecule (PECAM)-1 also participate in the structural integrity of AJs (18). Intracellularly, VE-Cadherin is linked to the actin cytoskeleton through its interactions with α-, β-, γ- and p120-Catenin (19) (Figure 1). In contrast, TJs are made by the homophilic interaction of cell adhesion molecules such as Claudins, Occludin and Junction Adhesion Molecules (JAMs), which are connected to the actin cytoskeleton through *Zona Occludens* (ZO)-1, -2 and -3 proteins (Figure 1). Because of their importance, the establishment and maintenance of cell-to-cell junctions are tightly controlled. One of the best illustrations of such regulation is the modulation of vessel permeability by the Vascular Endothelial Growth Factor (VEGF) (20, 21). Its binding to Vascular Endothelial Growth Factor

Receptor (VEGFR)-2 induces an increase in intracellular calcium levels, leading to the subsequent activation of Src family kinases, MAP kinases, PI3 kinase and protein kinase G (21, 22). This mainly results in *i)* the remodeling of the actin cytoskeleton, through the activation of the small GTPase Rho-A, *ii)* the activation of myosin light-chain kinase (MLCK) that favors actomyosin contractility, *iii)* the destabilization of integrin-mediated adhesion to the extracellular matrix and *iv)* the phosphorylation of VE-Cadherin and its internalization, thus loosening cell-cell junctions (23). All combined, these events participate in increasing endothelial permeability. In contrast, the activation of other small GTPases, such as Rac-1 or Cdc42, protect the barrier function of the endothelium by stabilizing intercellular junctions and the cortical actin cytoskeleton (24). Therefore, the fine regulation of the interface between intercellular junctions and the actin cytoskeleton plays a crucial role in regulating endothelial integrity.

Strikingly, certain pathogens have the ability to overcome the physical barrier imposed by the endothelium either from the outside towards the inside or/and vice versa to exit the vascular lumen and reach specific organs. Pathogenic bacteria can reach the circulation by accessing the vascular lumen through micro-abrasions within the skin or mucosa but also through insect bites (25-27). Once in the circulation, bacterial adhesion to the endothelium is a frequent starting point (28-30). Bacteria then either divert the host cell actin cytoskeleton to induce their internalization and transcytosis, leading to the passage of live bacteria through endothelial cells (31-35), or remain extracellular and interfere with the assembly of intercellular junctions facilitating their paracellular passage (36).

Hence, bacterial interaction with the endothelium often leads to the alteration of vascular integrity that might be at the origin of vascular leaks, bacterial dissemination within the surrounding tissues and/or organ dysfunction.

Throughout different examples of infection, we will here illustrate the many faces of bacteria-endothelium interactions and the subsequent perturbations of specific vascular functions in the particular environment of the blood circulation.

ALTERATION OF VASCULAR INTEGRITY UPON INFECTIONS BY *RICKETTSIA*

Spotted fevers associated with rickettsial infections are among the best characterized examples of pathogenic bacteria with a vascular tropism and disturbing endothelial functions. Members of the *Rickettsia* family are obligate intracellular vector-borne pathogens mainly transmitted by tick bites and triggering diverse diseases such as typhus or spotted fever (27). Endothelial cells of the peripheral circulation represent the main target of *Rickettsia* belonging to the spotted fever group (27, 37, 38) (Figure 2). *Rickettsia* adhesion to the endothelial surface is mediated by the expression of the outer-membrane protein (Omp)-A and B (39) and their interaction with endothelial integrins, such as the $\alpha_2\beta_1$ integrin (40). This induces a rapid and efficient internalization of the adherent bacteria within few minutes after the initial contact. Internalization occurs through a mechanism called "*induced phagocytosis*" that is at the crossroad between phagocytosis and endocytosis (41, 42) and involving Clathrin and Caveolin-2, two canonical proteins of the endocytic pathway (43).

Adhesion of *Rickettsia* onto endothelial cells also leads to a drastic remodeling of the actin cytoskeleton within the host cells that not only facilitates bacterial entry but also participates to bacteria movement and spreading within the endothelium. Endothelial cell surface-bond bacteria locally regulate actin rearrangements by recruiting the

Arp2/3 complex and activating Cdc42 and kinases of the Src-family to support bacterial internalization within phagosomal vesicles (44). The expression of the pore-forming proteins Hemolysin C and Phospholipase D by *Rickettsia* allows them to escape phagosomes and access the host cell cytosol (45-47) where they benefit from nutrients and energy present to support their growth (48). Within infected cells, *Rickettsia* also uses proteins from the actin cytoskeleton to propel and disseminate within adjacent endothelial cells. *Rickettsia* assembles polar actin tails made of unbranched parallel actin filaments, which help intracellular bacterial movement (49). The precise machinery allowing bacteria to assemble these actin comet tails remains debated. Whereas the involvement of RickA, a WASP-family protein homolog encoded by *Rickettsia*, in Arp2/3-mediated actin polymerization *in vitro* favors a mechanism of tail assembly relying on Arp2/3 activity (50, 51), Arp2/3 was not found to associate with *Rickettsia* actin tails (52, 53). An alternative hypothesis rather suggests that the bacterial protein Sca2 might participate in assembling actin tails through a formin-like mechanism (54, 55).

The infection of endothelial cells by *Rickettsia* leads to the activation of the endothelium, which is associated with the upregulation and secretion of a plethora of cytokines and chemokines, collectively referred to as *rickettsial vasculitis* (56). Interestingly, *Rickettsia* has developed different strategies to counteract immune responses and optimize their intracellular residence. First, *Rickettsia* has the ability to escape phagosomal vesicles before their fusion with lysosomes, hindering their degradation by the lysosomal content (47). Moreover, the bacterium activates the anti-apoptotic NF-κB signaling pathway within infected cells (57-60), thus balancing the killing of these cells mediated by the recruitment of CD8 T cells (61).

Importantly, *Rickettsia* also damages the endothelium by altering the assembly of endothelial intercellular junctions, most probably by disturbing the actin cytoskeleton (62), as well as by inducing an oxidative stress within infected cells that contributes to cell death (63, 64). Therefore, rickettsial infections lead to endothelial cell activation and dysfunction, including an alteration of the vascular integrity that results in the increase in vascular permeability and concurring to the pathophysiology of *Rickettsia*-induced vascular leaks (56, 65). Despite alterations in vascular integrity, rickettsial infections are not associated with subsequent dissemination to other organs, such as the brain as in the case of meningitis-causing pathogens.

VASCULAR COLONIZATION AND BLOOD-BRAIN BARRIER CROSSING BY *NEISSERIA MENINGITIDIS*

A limited number of pathogenic bacteria have developed mechanisms allowing them to cross the blood-brain barrier (BBB), most often triggering bacterial meningitis, a high-fatality rate disease (66). A hallmark feature of the clinical manifestations of bacterial meningitis is the presence of the pathogenic bacterium within the cerebrospinal fluid (CSF) where it triggers the inflammation of the meninges and the recruitment of immune cells within the CSF (66, 67). Since the bacterium is also found in the bloodstream, the most prevalent view is that the bacterium breaches the blood-CSF barrier to reach the CSF. However, as at this stage the anatomical site at which crossing occurs is not known, we will rather refer to crossing of the blood-brain barrier to be more inclusive.

With significant socioeconomic and geographic variations, *Neisseria meningitidis*, *Streptococcus pneumoniae* and type B *Haemophilus influenzae* are the most frequent causative agents of bacterial meningitis in adults and children. Of note,

while neonatal meningitis is mainly triggered by group B *Streptococcus*, *Listeria monocytogenes* and *Escherichia coli* K1 (68), immunocompromised patients frequently developed *Mycobacterium tuberculosis* or non-typhoid *Salmonella* meningitis (69, 70). Despite their heterogeneity, common themes in the mechanisms used by these pathogenic bacteria to cross the BBB can be drawn (for review, see (71)).

Recent studies have pointed to the importance of a process called *vascular colonization* during *N. meningitidis* infections and probably during meningitis (72). This bacterium has the ability to adhere to the endothelial surface and proliferate in the form of bacterial aggregates that eventually fill the lumen of small vessels of 10 to 50 µm in diameter (Figure 3). A recent study shows that this colonization process is facilitated by the honey-like viscous liquid properties of the bacterial aggregates which allow them to adapt to the complex morphology of the vasculature upon proliferation (73). Adhesion to the endothelium likely participates in the immune evasion as this prevents phagocytosis from Küpffer cells in the liver. Histological analysis of *post-mortem* samples reveals large bacterial aggregates in the brain vessels suggesting that vascular colonization could promote BBB crossing by concentrating a high number of bacteria at specific sites and by altering endothelial cell physiology (74). The combined effect of vessel occlusion due to bacterial accumulation and the activation of the coagulation cascade (75) might participate in altering endothelium integrity.

Alternatively, *in vitro* studies have identified specific signaling pathways triggered by *N. meningitidis*, which lead to the opening of intercellular junctions within the cerebral endothelium. Meningococcal adhesion to the host cells is mediated by their expression of type four pili (Tfp) (76) that engage host cell surface receptors, such as

CD147 and β2-adrenergic receptor (77, 78). While proliferating at the surface of infected endothelial cells, thus forming bacterial aggregates, meningococci induce the remodeling of the host cell plasma membrane (79, 80) (Figure 3). Associated to their aggregation capacity, plasma membrane protrusions infiltrating meningococcal microcolonies were shown to enhance the mechanical cohesion of the microcolony thus allowing them to resist the blood-flow induced shear stress (81). Interestingly, although the cortical actin network is strongly reorganized below the bacterial colonies (82), the active contribution of the host cells has been shown to be dispensable in the *Nm*-mediated plasma membrane remodeling. The actin cytoskeleton or even the intracellular ATP is not necessary for the Tfp-induced plasma membrane reorganization (80, 81). Upon bacterial adhesion to microvascular endothelial cells, Tfp trigger a complex cascade of signaling events leading to the formation of “ectopic junctions” underneath bacterial aggregates together with the local remodeling of the cortical actin cytoskeleton (83). This process relies, on the one hand, on the activation of small GTPases such as cell division cycle protein 42 (Cdc42) and Rac1, along with the local recruitment of proteins of the polarity complex, including partitioning-defective 3 (PAR3), PAR6 and protein kinase C-ζ (PKC-ζ), as well as the branched-actin nucleating complex Arp2/3. On the other hand, by mistargeting recycling endosomes, meningococci induce the accumulation of junctional proteins, such as VE-Cadherin, underneath bacterial aggregates, a process shown to weaken intercellular junctions and increase blood-brain barrier permeability, thus facilitating meningococcal dissemination within the cerebral tissues and the cerebrospinal fluid (CSF) (83). These elaborated mechanisms illustrate the panoply of strategies enabling pathogenic bacteria to alter the endothelial barrier.

ALTERATION OF THE ENDOCARDIUM AND ENDOCARDITIS

Infective endocarditis (IE) is a bacterial infection of the cardiac endothelium. The hallmark of IE is the colonization and destruction of the cardiac valves by pathogenic bacteria following local endothelial injury or inflammation (84, 85) (for review, see (86)). Although the causative agent of such a disease greatly varies according to the geographic zones, most IE result from *Staphylococcus aureus*, *Enteroccocus* or *Streptococcus* species, among which *S. gallolyticus* (87, 88).

The pathologic cascade starts following sterile lesions of the cardiac valve endothelium of unclear origin that lead to the exposure of the extracellular matrix. This triggers the formation of a platelet- and fibrin-rich thrombus, considered as a hot-spot for the adhesion of blood-circulating bacteria (89) (Figure 4). Alternatively, bacterial adhesion can occur at the surface of inflamed endothelium, a process facilitated by the local upregulation of cell surface adhesion molecules, such as β1 integrins (90). From the bacterial side, adhesion is mediated by the surface expression of extracellular matrix-targeting adhesins, such as fibronectin binding proteins (FnBPs) (91, 92). Adherent bacteria locally proliferate and form a *vegetation*, a biofilm-like structure where aggregated bacteria are mixed together with extracellular matrix proteins, clot components and/or immune cells (93). While the vegetation matures, the adjacent endothelial cells are exposed, thus driving the propagation of the local inflammation and cell death and ultimately leading to the destruction of the infected valves thus requiring surgical replacement (94). However, this mechanism probably does not entirely account for IE induced by intracellular bacteria such as *Bartonella* species or *Staphylococcus aureus*, which rather rely on the secretion of exoenzymes and toxins to mediate their pathogenic effects (95) and for which the host immune response might play an important role (96). Although

Bartonella species have been described in relatively rare cases of human endocarditis (97, 98) with a preferential localization at the aortic valve (99), these bacteria are mostly known for their involvement in angioproliferative syndromes.

ANGIOPROLIFERATION DURING *BARTONELLA* INFECTIONS

As mentioned earlier, angiogenesis supports the formation of new blood vessels from pre-existing ones (12). Interestingly, this process can be diverted by pathogenic bacteria and especially *Bartonella henselae* (for review, see (100)). *Bartonella spp.* are Gram-negative organisms found in domestic and wild mammals with a tropism for red blood cells and endothelial cells (100, 101). While in healthy individuals, *Bartonella henselae* infections cause benign cat scratch diseases (102), in immunocompromised patients these infections can trigger a vasoproliferative syndrome resulting in the formation of tumor-like nodules in the skin, known as cutaneous bacillary angiomatosis (BA) (100). This results from the ability of *Bartonella henselae* to invade endothelial cells and trigger their proliferation and migration (103, 104) together with the recruitment of macrophages, monocytes and polymorphonuclear neutrophils (105, 106) (Figure 5).

Interestingly, similar to *Neisseria meningitidis* microcolonies, *B. henselae* also forms plasma membrane-associated bacterial aggregates that either remain at the surface of or are internalized in infected endothelial cells (106). Two actin-dependent mechanisms have been described regarding bacterial internalization within endothelial cells (107, 108): the first one is reminiscent of the previously described bacterium-induced phagocytosis and allows the relatively fast entry of *Bartonella* within perinuclear phagosomes (109). The second mechanism, lasting for up to 24 hours, allows the slow internalization of small *B. henselae* aggregates within large

vacuoles, referred to as invasomes (107). Of note, similarly to the protective mechanisms developed by *Rickettsia* to promote their survival during their intracellular residence, *Bartonella* is able to inhibit key steps of the apoptosis program induced upon cell infections (110).

Although not fully understood, the proliferation of infected endothelial cells is in part supported by bacterial proteins that are translocated within the host cells through the VirB-VirD4 type IV secretion system (T4SS) encoded by *Bartonella* (111, 112). In addition, several reports have shown that macrophages, locally recruited upon endothelial cell infection, participate in the pathological angiogenesis induced by *Bartonella*. Indeed, macrophages are well-known producers of pro-angiogenic factors upon activation (113). *In vitro*, macrophages have been shown to support endothelial proliferation through the secretion of VEGF in response to *B. henselae* infection (114, 115), thus suggesting that macrophages are involved in a paracrine loop that enhances *Bartonella*-mediated vasoproliferation (116).

SYSTEMIC IMPLICATIONS OF VASCULAR INFECTIONS

Under steady-state conditions, besides normal transitory bacteremia, the vascular organ is thought to be sterile (26). Therefore, it possesses robust mechanisms to recognize circulating pathogens and trigger innate and/or adaptive immune responses (117, 118). Similarly to immune cells specialized in pathogen recognition, endothelial cells express chemokine receptors, such as CXCR-1, -2 and -4 (119, 120) as well as pattern-recognition receptors (PRRs), such as Toll-Like Receptors (TLRs) and NOD-Like Receptors (NLRs) (121, 122). They have also been shown to secrete pro-inflammatory molecules, such as the cytokines interleukin (IL)-1 and -8 (123-125) and to respond to bacterial lipopolysaccharide (LPS), tumor necrosis

factor-α (TNF-α) or interferon-γ (INF-γ) by signaling through the canonical pro-inflammatory NF-κB pathway (126). Hence, the endothelium possesses an array of tools allowing the recognition of pathogenic microorganisms and the recruitment of cells from the innate immune system in order to clear blood-circulating pathogens.

Of particular interest, endothelial cells (ECs) also participate in mounting adaptive immune responses since they can act as antigen presenting cells (APCs) (127). The hallmark of APCs is their expression of major histocompatibility complex class II (MHC-II) molecules allowing them to present extracellular antigens to T cells (128). Whereas quiescent ECs express basal levels of MHC-II molecules (129), they possess the capacity to upregulate their expression upon activation (130), providing them with the ability to present antigenic determinants to T cells and rapidly initiate pathogen-specific immune responses. Therefore, endothelial cells are equipped to recognize pathogenic microorganisms, locally attract cells of the innate immunity and serve as a link to trigger adaptive immune responses in order to efficiently fight invaders (for review, see (131)).

Although invasion of the endothelium by bacteria leads to the activation of the immune system, in absence of appropriate treatments or when the body fails clearing the pathogens, it might evolve toward an uncontrolled and systemic affection. Indeed, the constant release of damage-associated molecular patterns (DAMPs) by invading bacteria and/or injured endothelium leads to the imbalance of various body systems, among which the overstimulation of immune cells through TLRs and the complement pathway (132), as well as the exacerbated production of cytokines, referred to as the *cytokine storm* (133). Together with a persistent bacteremia, this systemic inflammatory response syndrome (SIRS) is the hallmark of sepsis (134, 135).

Paradoxically, a common feature of sepsis is its association with a form of immune suppression occurring after the unregulated inflammation (132, 136). While not fully understood, sepsis is marked by the severe depletion of T and B cells, as well as dendritic cells, which all show an enhanced pro-apoptotic activity (137, 138). In addition, in patients suffering from sepsis, a bias in the ratio of regulatory over effector T cells is often observed (138, 139). The latter also showing a reduced ability to produce cytokines, a feature known as T cell exhaustion (140), most probably due to dysregulations in the programmed cell death 1 (PD1) - PD1 ligand 1 (PDL1) axis (141), owing to the exacerbated cytokine production.

In addition, sepsis frequently affects the coagulation pathway: ranging from the formation of small thrombi to the manifestation of disseminated intravascular coagulation (DIC) –which corresponds to the coagulation of the blood throughout the entire body– coagulopathies are one of the major complications in sepsis and have been extensively reviewed elsewhere (142, 143). Nevertheless, we would like to mention that perturbation of the coagulation pathway occurs early during sepsis and first results from the activation of the endothelium in response to the cytokine storm, thus favoring the local deposition of fibrin at the surface of the vessel walls (133).

Alternatively, endotoxins derived from Gram-negative bacteria, such as the lipopolysaccharide (LPS), can trigger in a NF-κB-dependent manner both the secretion and the surface expression of tissue factor (TF) by endothelial cells and circulatory blood cells. By making a complex with the activated coagulation factor VII (FVIIa), TF is a highly potent pro-coagulant molecule (144, 145). In both scenarios, thrombus formation in turn leads to the activation of the endothelial cell surface protease-activated receptor (PAR)-1 that signals through the small GTPase RhoA to disassemble actin filaments and induce VE-Cadherin internalization, hence affecting

the stability of intercellular junctions and the integrity of the vascular endothelium (118). As a consequence, vessels become leaky, blood pressure decreases and proteins from the endothelial extracellular matrix, such as collagen, are exposed to the vessel content, which further activates platelet aggregation and fibrin formation (118, 143). Multi-organ failure is often associated with the late phases of DIC, which results from microvascular thrombosis and poor tissue perfusion (146, 147).

CONCLUDING REMARKS

Bacterial infections taking place in the circulation are particularly problematic because of the specific alterations they cause to the circulation, possibly affecting the entire body. According to the specific site of infection and the properties of the different pathogens, a complex set of interactions takes place during these infections. Blood vessels are highly diverse with broad ranges of size and structure and each pathogen has a set of virulence factors that alter blood vessel function in specific ways. As a result, clinical manifestations are also very different. However, despite this diversity, the endothelium is at the center of these infectious processes and a limited number of endothelial functions are targeted in these infectious contexts: the integrity of the vasculature and its permeability, but also its inflammatory and coagulation status. More research is needed on host-pathogen interactions during these systemic infections and on endothelial cell biology to better treat these infections.

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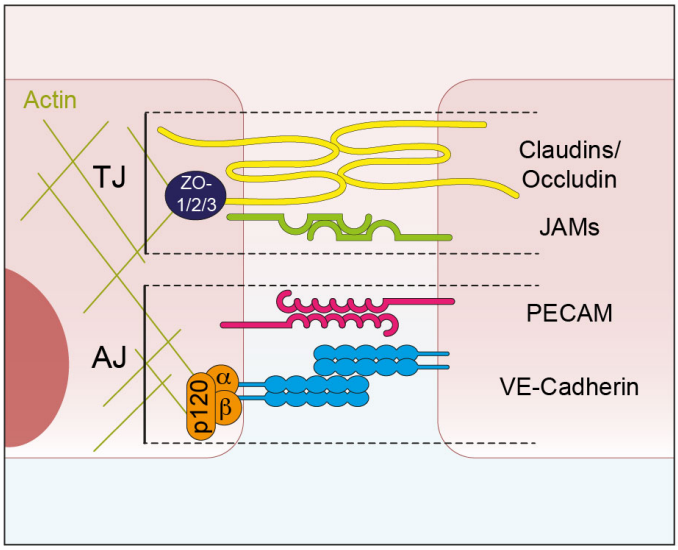
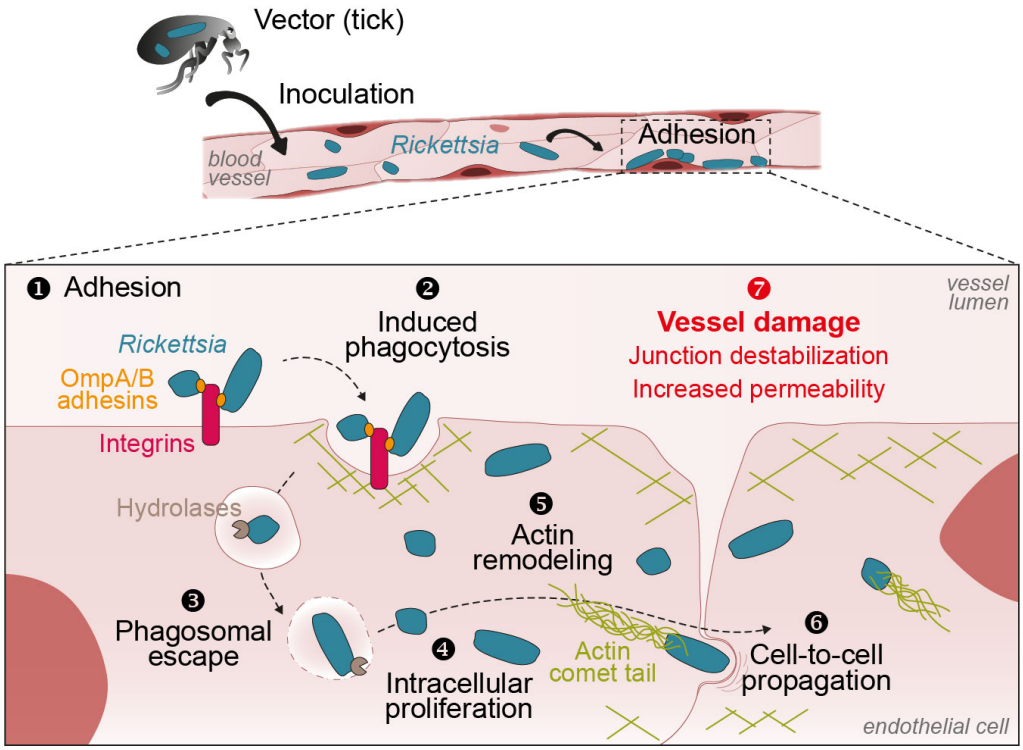


Figure 1: Schematic representation of the two main types of intercellular junctions within the endothelium. *Adherens* junctions (AJ) are made by the homophilic interaction of Vascular Endothelial (VE)-Cadherin and PECAM (Platelet endothelial cell adhesion molecule, also known as CD31). In contrast, claudins, occludin and proteins from the junctional adhesion molecules (JAMs) family are involved in establishing tight junctions. Connection with the actin cytoskeleton is ensured by proteins of the catenin family (alpha-, beta- and p120-catenin) in the case of *adherens* junctions, and proteins from the zonula occludens family (ZO-1, -2 and -3) in the case of tight junctions.



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842 **Figure 2: Infection of the endothelium by *Rickettsia*.** Following bacterial
843 inoculation into the lumen of blood vessels, *Rickettsia* adheres at the surface of the
844 endothelium through the surface expression of the outer-membrane protein (Omp)-A
845 and -B. Binding of Omp-A/B to cell-surface integrins induces the phagocytosis of
846 bacteria and the remodeling of the cellular actin cytoskeleton. Then, Hemolysin C
847 and/or Phospholipase D-expressing bacteria escape phagosomal vesicles, proliferate
848 intracellularly and utilize cellular components, such as actin monomers and nutrients,
849 to assemble actin comet tails supporting bacterial movement and cell-to-cell
850 spreading. Both actin cytoskeleton remodeling and bacterial propagation participate
851 in damaging infected vessels, including the destabilization of cellular junctions
852 responsible for the increase in vessel permeability.

853

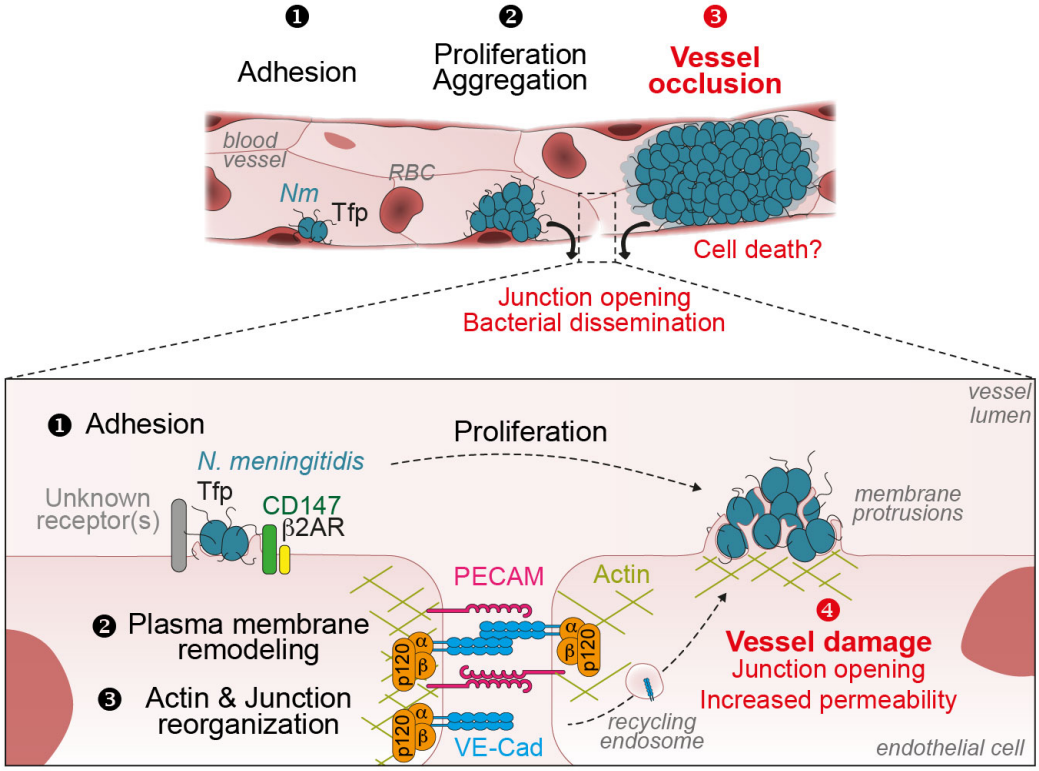
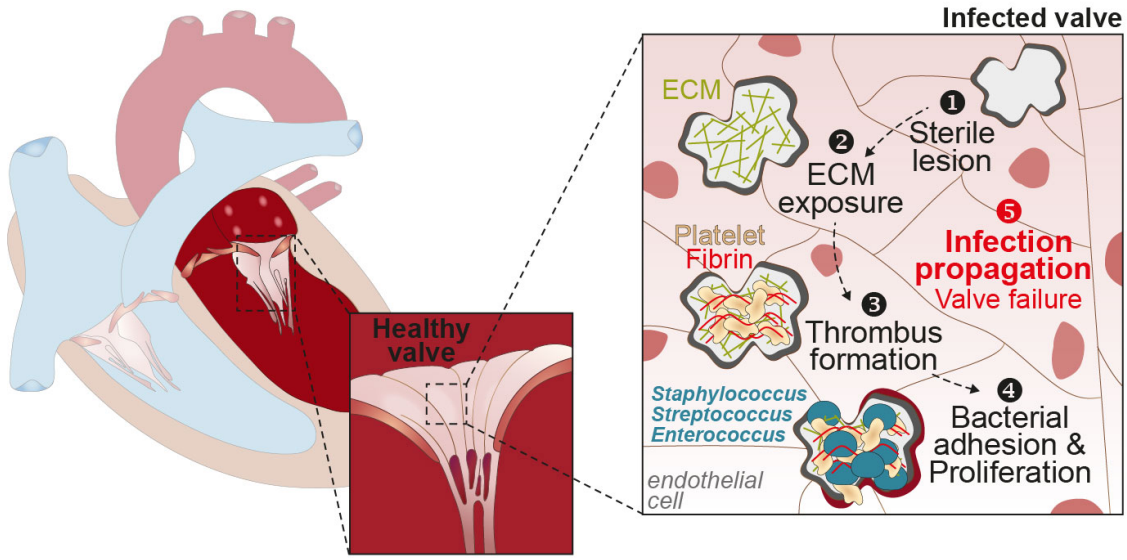


Figure 3: Vascular colonization by *Neisseria meningitidis*. Once into the bloodstream, *Neisseria meningitidis* adheres to the endothelium thanks to the surface expression of type four pili (Tfp). While proliferating, and owing to their auto-aggregative property, bacteria form a tight microcolony at the surface of the endothelium, which ultimately leads to the congestion of the colonized vessel. Bacterial adhesion at the surface of endothelial cells induces a drastic remodeling of the host cell-plasma membrane that forms membrane protrusions interdigitating within the bacterial aggregate. In addition, pilus interaction with endothelial cell-surface receptors, such as CD147 or β2-adrenergic receptor (β2-AR), induces the reorganization of the actin cytoskeleton and intercellular junctions by recruiting their components underneath the microcolony. Taken together, these events are proposed to destabilize intercellular junctions, hence resulting in the increase in vessel permeability.



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870 **Figure 4: The stepwise process leading to endocarditis.** The apparition of sterile
871 lesions (most often of unknown origin) on the heart valvular endothelium leads to the
872 exposure of the underneath extracellular matrix (ECM). This in turn triggers the
873 formation of a thrombus – characterized by the local deposition of platelets and fibrin
874 at the surface of the damaged endothelium – that favors bacterial adhesion. While
875 bacteria proliferate and spread, the valvular endothelium become more and more
876 damaged, eventually leading to the failure of the valve and the need for its surgical
877 replacement.

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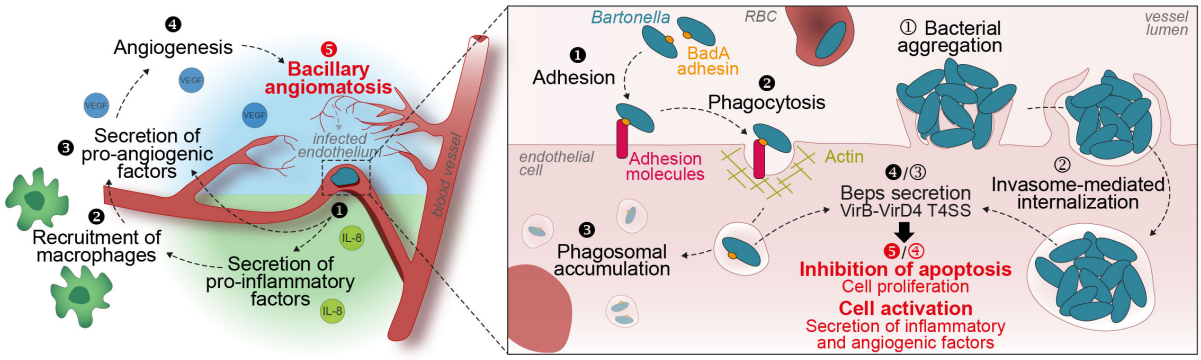

Figure 5: *Bartonella*-induced angioproliferation. Interactions of *Bartonella* with the endothelium might occur at the single-bacterium level through the bacterial expression of the Bartonella adhesin A (BadA) protein. This triggers the phagocytosis of the cell-surface bound bacteria and results in their perinuclear accumulation within phagosomes. Similarly, to *Neisseria meningitidis*, *Bartonella* also forms aggregates that are internalized through a slower process within big vacuoles, referred to as invasomes. In both cases, the VirB-VirD4 type four secretion system (T4SS)-dependent cytoplasmic release of *Bartonella* effector proteins (Beps) by intra-vesicular bacteria promotes the proliferation and activation of the infected endothelial cells. This notably results in the secretion by the endothelium of pro-inflammatory (*e.g.* IL-8) and pro-angiogenic (*e.g.* VEGF) factors. As a consequence, cells from the innate immunity, including neutrophils and macrophages, are locally recruited to fight the infection. Activated macrophages locally secrete VEGF, thus reinforcing the pro-angiogenic microenvironment. Combined to the bacterium-mediated endothelial cell proliferation, this particular environment promotes angiogenesis that ultimately leads to the local accumulation of new blood capillaries and the formation of Bacillary Angiomatosis lesions.