The many faces of bacterium-endothelium interactions during systemic infections

Dorian Obino and Guillaume Duménil*

Affiliation

Pathogenesis of Vascular Infections, Institut Pasteur, INSERM, Paris, France.

* Correspondence: guillaume.dumenil@pasteur.fr

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 Kpffer 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-bound bacteria locally regulate actin rearrangements by recruiting the

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

161 The infection of endothelial cells by *Rickettsia* leads to the activation of the
162 endothelium, which is associated with the upregulation and secretion of a plethora of
163 cytokines and chemokines, collectively referred to as *rickettsial vasculitis* (56).
164 Interestingly, *Rickettsia* has developed different strategies to counteract immune
165 responses and optimize their intracellular residence. First, *Rickettsia* has the ability to
166 escape phagosomal vesicles before their fusion with lysosomes, hindering their
167 degradation by the lysosomal content (47). Moreover, the bacterium activates the
168 anti-apoptotic NF- κ B signaling pathway within infected cells (57-60), thus balancing
169 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 Kpffer 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 β 2-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*, *Enterococcus* 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 $\beta 1$ 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

320 factor- α (TNF- α) or interferon- γ (INF- γ) by signaling through the canonical pro-
321 inflammatory NF- κ B pathway (126). Hence, the endothelium possesses an array of
322 tools allowing the recognition of pathogenic microorganisms and the recruitment of
323 cells from the innate immune system in order to clear blood-circulating pathogens.
324 Of particular interest, endothelial cells (ECs) also participate in mounting adaptive
325 immune responses since they can act as antigen presenting cells (APCs) (127). The
326 hallmark of APCs is their expression of major histocompatibility complex class II
327 (MHC-II) molecules allowing them to present extracellular antigens to T cells (128).
328 Whereas quiescent ECs express basal levels of MHC-II molecules (129), they
329 possess the capacity to upregulate their expression upon activation (130), providing
330 them with the ability to present antigenic determinants to T cells and rapidly initiate
331 pathogen-specific immune responses. Therefore, endothelial cells are equipped to
332 recognize pathogenic microorganisms, locally attract cells of the innate immunity and
333 serve as a link to trigger adaptive immune responses in order to efficiently fight
334 invaders (for review, see (131)).

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

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

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

361 Alternatively, endotoxins derived from Gram-negative bacteria, such as the
362 lipopolysaccharide (LPS), can trigger in a NF- κ B-dependent manner both the
363 secretion and the surface expression of tissue factor (TF) by endothelial cells and
364 circulatory blood cells. By making a complex with the activated coagulation factor VII
365 (FVIIa), TF is a highly potent pro-coagulant molecule (144, 145). In both scenarios,
366 thrombus formation in turn leads to the activation of the endothelial cell surface
367 protease-activated receptor (PAR)-1 that signals through the small GTPase RhoA to
368 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.

394 **Acknowledgments**

395 The authors acknowledge Daria Bonazzi and Paul Kennouche for the critical reading
396 of the manuscript. DO was supported by a Pasteur-Roux postdoctoral fellowship from
397 the Institut Pasteur. Funding was obtained from the European Research Council
398 (ERC VIP consolidator grant) and the Integrative Biology of Emerging Infectious
399 Diseases (IBEID) laboratory of excellence (ANR-10-LABX-62) to GD.

References

1. **Lerman A, Burnett JC, Jr.** 1992. Intact and altered endothelium in regulation of vasomotion. *Circulation* **86**:III12-19.
2. **Sender R, Fuchs S, Milo R.** 2016. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol* **14**:e1002533.
3. **Pugsley MK, Tabrizchi R.** 2000. The vascular system. An overview of structure and function. *J Pharmacol Toxicol Methods* **44**:333-340.
4. **Brozovich FV, Nicholson CJ, Degen CV, Gao YZ, Aggarwal M, Morgan KG.** 2016. Mechanisms of Vascular Smooth Muscle Contraction and the Basis for Pharmacologic Treatment of Smooth Muscle Disorders. *Pharmacol Rev* **68**:476-532.
5. **Galili O, Herrmann J, Woodrum J, Sattler KJ, Lerman LO, Lerman A.** 2004. Adventitial vasa vasorum heterogeneity among different vascular beds. *J Vasc Surg* **40**:529-535.
6. **Sarin H.** 2010. Physiologic upper limits of pore size of different blood capillary types and another perspective on the dual pore theory of microvascular permeability. *J Angiogenes Res* **2**:14.
7. **Bilzer M, Roggel F, Gerbes AL.** 2006. Role of Kupffer cells in host defense and liver disease. *Liver Int* **26**:1175-1186.
8. **Harris IS, Black BL.** 2010. Development of the endocardium. *Pediatr Cardiol* **31**:391-399.
9. **Carmeliet P.** 2003. Angiogenesis in health and disease. *Nat Med* **9**:653-660.
10. **Zecchin A, Kalucka J, Dubois C, Carmeliet P.** 2017. How Endothelial Cells Adapt Their Metabolism to Form Vessels in Tumors. *Front Immunol* **8**:1750.
11. **Risau W, Flamme I.** 1995. Vasculogenesis. *Annu Rev Cell Dev Biol* **11**:73-91.
12. **Risau W.** 1997. Mechanisms of angiogenesis. *Nature* **386**:671-674.
13. **Diaz-Santana A, Shan M, Stroock AD.** 2015. Endothelial cell dynamics during anastomosis in vitro. *Integr Biol (Camb)* **7**:454-466.
14. **Komarova Y, Malik AB.** 2010. Regulation of endothelial permeability via paracellular and transcellular transport pathways. *Annu Rev Physiol* **72**:463-493.
15. **Hawkins BT, Davis TP.** 2005. The blood-brain barrier/neurovascular unit in health and disease. *Pharmacol Rev* **57**:173-185.
16. **Daneman R, Prat A.** 2015. The blood-brain barrier. *Cold Spring Harb Perspect Biol* **7**:a020412.
17. **Rudini N, Dejana E.** 2008. Adherens junctions. *Curr Biol* **18**:R1080-1082.
18. **Privratsky JR, Newman PJ.** 2014. PECAM-1: regulator of endothelial junctional integrity. *Cell Tissue Res* **355**:607-619.
19. **Hartsock A, Nelson WJ.** 2008. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochim Biophys Acta* **1778**:660-669.
20. **Bates DO, Harper SJ.** 2002. Regulation of vascular permeability by vascular endothelial growth factors. *Vascul Pharmacol* **39**:225-237.
21. **Bates DO.** 2010. Vascular endothelial growth factors and vascular permeability. *Cardiovasc Res* **87**:262-271.
22. **Wu HM, Huang Q, Yuan Y, Granger HJ.** 1996. VEGF induces NO-dependent hyperpermeability in coronary venules. *Am J Physiol* **271**:H2735-2739.

23. **Aghajanian A, Wittchen ES, Allingham MJ, Garrett TA, Burridge K.** 2008. Endothelial cell junctions and the regulation of vascular permeability and leukocyte transmigration. *J Thromb Haemost* **6**:1453-1460.
24. **Popoff MR, Geny B.** 2009. Multifaceted role of Rho, Rac, Cdc42 and Ras in intercellular junctions, lessons from toxins. *Biochim Biophys Acta* **1788**:797-812.
25. **Round H, Kirkpatrick HJ, Hails CG.** 1936. Further Investigations on Bacteriological Infections of the Mouth: (Section of Odontology). *Proc R Soc Med* **29**:1552-1556.
26. **Cobe HM.** 1954. Transitory bacteremia. *Oral Surg Oral Med Oral Pathol* **7**:609-615.
27. **Snowden J, Bhimji SS.** 2018. Rickettsial Infection, *StatPearls*, Treasure Island (FL).
28. **Stins MF, Prasadarao NV, Ibric L, Wass CA, Luckett P, Kim KS.** 1994. Binding characteristics of S fimbriated Escherichia coli to isolated brain microvascular endothelial cells. *Am J Pathol* **145**:1228-1236.
29. **Greiffenberg L, Goebel W, Kim KS, Weiglein I, Bubert A, Engelbrecht F, Stins M, Kuhn M.** 1998. Interaction of Listeria monocytogenes with human brain microvascular endothelial cells: InlB-dependent invasion, long-term intracellular growth, and spread from macrophages to endothelial cells. *Infect Immun* **66**:5260-5267.
30. **Sheen TR, Ebrahimi CM, Hiemstra IH, Barlow SB, Peschel A, Doran KS.** 2010. Penetration of the blood-brain barrier by Staphylococcus aureus: contribution of membrane-anchored lipoteichoic acid. *J Mol Med (Berl)* **88**:633-639.
31. **Stins MF, Badger J, Sik Kim K.** 2001. Bacterial invasion and transcytosis in transfected human brain microvascular endothelial cells. *Microb Pathog* **30**:19-28.
32. **Jain SK, Paul-Satyaseela M, Lamichhane G, Kim KS, Bishai WR.** 2006. Mycobacterium tuberculosis invasion and traversal across an in vitro human blood-brain barrier as a pathogenic mechanism for central nervous system tuberculosis. *J Infect Dis* **193**:1287-1295.
33. **Nikulin J, Panzner U, Frosch M, Schubert-Unkmeir A.** 2006. Intracellular survival and replication of Neisseria meningitidis in human brain microvascular endothelial cells. *Int J Med Microbiol* **296**:553-558.
34. **Prasadarao NV, Wass CA, Stins MF, Shimada H, Kim KS.** 1999. Outer membrane protein A-promoted actin condensation of brain microvascular endothelial cells is required for Escherichia coli invasion. *Infect Immun* **67**:5775-5783.
35. **Das A, Asatryan L, Reddy MA, Wass CA, Stins MF, Joshi S, Bonventre JV, Kim KS.** 2001. Differential role of cytosolic phospholipase A2 in the invasion of brain microvascular endothelial cells by Escherichia coli and Listeria monocytogenes. *J Infect Dis* **184**:732-737.
36. **Dumenil G.** 2011. Revisiting the extracellular lifestyle. *Cell Microbiol* **13**:1114-1121.
37. **Olano JP.** 2005. Rickettsial infections. *Ann N Y Acad Sci* **1063**:187-196.
38. **Walker DH, Ismail N.** 2008. Emerging and re-emerging rickettsioses: endothelial cell infection and early disease events. *Nat Rev Microbiol* **6**:375-386.

39. **Li H, Walker DH.** 1998. rOmpA is a critical protein for the adhesion of *Rickettsia rickettsii* to host cells. *Microb Pathog* **24**:289-298.
40. **Hillman RD, Jr., Baktash YM, Martinez JJ.** 2013. OmpA-mediated rickettsial adherence to and invasion of human endothelial cells is dependent upon interaction with alpha2beta1 integrin. *Cell Microbiol* **15**:727-741.
41. **Walker TS, Winkler HH.** 1978. Penetration of cultured mouse fibroblasts (L cells) by *Rickettsia prowazekii*. *Infect Immun* **22**:200-208.
42. **Walker TS.** 1984. Rickettsial interactions with human endothelial cells in vitro: adherence and entry. *Infect Immun* **44**:205-210.
43. **Chan YG, Cardwell MM, Hermanas TM, Uchiyama T, Martinez JJ.** 2009. Rickettsial outer-membrane protein B (rOmpB) mediates bacterial invasion through Ku70 in an actin, c-Cbl, clathrin and caveolin 2-dependent manner. *Cell Microbiol* **11**:629-644.
44. **Martinez JJ, Cossart P.** 2004. Early signaling events involved in the entry of *Rickettsia conorii* into mammalian cells. *J Cell Sci* **117**:5097-5106.
45. **Radulovic S, Troyer JM, Beier MS, Lau AO, Azad AF.** 1999. Identification and molecular analysis of the gene encoding *Rickettsia typhi* hemolysin. *Infect Immun* **67**:6104-6108.
46. **Renesto P, Dehoux P, Gouin E, Touqui L, Cossart P, Raoult D.** 2003. Identification and characterization of a phospholipase D-superfamily gene in rickettsiae. *J Infect Dis* **188**:1276-1283.
47. **Whitworth T, Popov VL, Yu XJ, Walker DH, Bouyer DH.** 2005. Expression of the *Rickettsia prowazekii* pld or tlyC gene in *Salmonella enterica* serovar Typhimurium mediates phagosomal escape. *Infect Immun* **73**:6668-6673.
48. **Walker DH, Valbuena GA, Olano JP.** 2003. Pathogenic mechanisms of diseases caused by *Rickettsia*. *Ann N Y Acad Sci* **990**:1-11.
49. **Heinzen RA.** 2003. Rickettsial actin-based motility: behavior and involvement of cytoskeletal regulators. *Ann N Y Acad Sci* **990**:535-547.
50. **Jeng RL, Goley ED, D'Alessio JA, Chaga OY, Svitkina TM, Borisy GG, Heinzen RA, Welch MD.** 2004. A *Rickettsia* WASP-like protein activates the Arp2/3 complex and mediates actin-based motility. *Cell Microbiol* **6**:761-769.
51. **Gouin E, Egile C, Dehoux P, Villiers V, Adams J, Gertler F, Li R, Cossart P.** 2004. The RickA protein of *Rickettsia conorii* activates the Arp2/3 complex. *Nature* **427**:457-461.
52. **Gouin E, Gantelet H, Egile C, Lasa I, Ohayon H, Villiers V, Gounon P, Sansonetti PJ, Cossart P.** 1999. A comparative study of the actin-based motilities of the pathogenic bacteria *Listeria monocytogenes*, *Shigella flexneri* and *Rickettsia conorii*. *J Cell Sci* **112** (Pt 11):1697-1708.
53. **Harlander RS, Way M, Ren Q, Howe D, Grieshaber SS, Heinzen RA.** 2003. Effects of ectopically expressed neuronal Wiskott-Aldrich syndrome protein domains on *Rickettsia rickettsii* actin-based motility. *Infect Immun* **71**:1551-1556.
54. **Haglund CM, Choe JE, Skau CT, Kovar DR, Welch MD.** 2010. *Rickettsia* Sca2 is a bacterial formin-like mediator of actin-based motility. *Nat Cell Biol* **12**:1057-1063.
55. **Kleba B, Clark TR, Lutter EI, Ellison DW, Hackstadt T.** 2010. Disruption of the *Rickettsia rickettsii* Sca2 autotransporter inhibits actin-based motility. *Infect Immun* **78**:2240-2247.
56. **Sahni SK, Narra HP, Sahni A, Walker DH.** 2013. Recent molecular insights into rickettsial pathogenesis and immunity. *Future Microbiol* **8**:1265-1288.

57. **Sporn LA, Sahni SK, Lerner NB, Marder VJ, Silverman DJ, Turpin LC, Schwab AL.** 1997. Rickettsia rickettsii infection of cultured human endothelial cells induces NF-kappaB activation. *Infect Immun* **65**:2786-2791.
58. **Sahni SK, Van Antwerp DJ, Eremeeva ME, Silverman DJ, Marder VJ, Sporn LA.** 1998. Proteasome-independent activation of nuclear factor kappaB in cytoplasmic extracts from human endothelial cells by Rickettsia rickettsii. *Infect Immun* **66**:1827-1833.
59. **Clifton DR, Goss RA, Sahni SK, van Antwerp D, Baggs RB, Marder VJ, Silverman DJ, Sporn LA.** 1998. NF-kappa B-dependent inhibition of apoptosis is essential for host cell survival during Rickettsia rickettsii infection. *Proc Natl Acad Sci U S A* **95**:4646-4651.
60. **Walker DH.** 2007. Rickettsiae and rickettsial infections: the current state of knowledge. *Clin Infect Dis* **45 Suppl 1**:S39-44.
61. **Walker DH, Olano JP, Feng HM.** 2001. Critical role of cytotoxic T lymphocytes in immune clearance of rickettsial infection. *Infect Immun* **69**:1841-1846.
62. **Valbuena G, Walker DH.** 2005. Changes in the adherens junctions of human endothelial cells infected with spotted fever group rickettsiae. *Virchows Arch* **446**:379-382.
63. **Santucci LA, Gutierrez PL, Silverman DJ.** 1992. Rickettsia rickettsii induces superoxide radical and superoxide dismutase in human endothelial cells. *Infect Immun* **60**:5113-5118.
64. **Eremeeva ME, Silverman DJ.** 1998. Effects of the antioxidant alpha-lipoic acid on human umbilical vein endothelial cells infected with Rickettsia rickettsii. *Infect Immun* **66**:2290-2299.
65. **Schmaier AH, Srikanth S, Elghetany MT, Normolle D, Gokhale S, Feng HM, Walker DH.** 2001. Hemostatic/fibrinolytic protein changes in C3H/HeN mice infected with Rickettsia conorii--a model for Rocky Mountain spotted fever. *Thromb Haemost* **86**:871-879.
66. **Doran KS, Fulde M, Gratz N, Kim BJ, Nau R, Prasadarao N, Schubert-Unkmeir A, Tuomanen EI, Valentin-Weigand P.** 2016. Host-pathogen interactions in bacterial meningitis. *Acta Neuropathol* **131**:185-209.
67. **Brandtzaeg P, van Deuren M.** 2012. Classification and pathogenesis of meningococcal infections. *Methods Mol Biol* **799**:21-35.
68. **Thigpen MC, Whitney CG, Messonnier NE, Zell ER, Lynfield R, Hadler JL, Harrison LH, Farley MM, Reingold A, Bennett NM, Craig AS, Schaffner W, Thomas A, Lewis MM, Scallan E, Schuchat A, Emerging Infections Programs N.** 2011. Bacterial meningitis in the United States, 1998-2007. *N Engl J Med* **364**:2016-2025.
69. **Bekondi C, Bernede C, Passone N, Minssart P, Kamalo C, Mbolidi D, Germani Y.** 2006. Primary and opportunistic pathogens associated with meningitis in adults in Bangui, Central African Republic, in relation to human immunodeficiency virus serostatus. *Int J Infect Dis* **10**:387-395.
70. **Fernandez Guerrero ML, Ramos JM, Nunez A, Cuenca M, de Gorgolas M.** 1997. Focal infections due to non-typhi Salmonella in patients with AIDS: report of 10 cases and review. *Clin Infect Dis* **25**:690-697.
71. **van Sorge NM, Doran KS.** 2012. Defense at the border: the blood-brain barrier versus bacterial foreigners. *Future Microbiol* **7**:383-394.
72. **Melican K, Michea Veloso P, Martin T, Bruneval P, Dumenil G.** 2013. Adhesion of Neisseria meningitidis to dermal vessels leads to local vascular

- damage and purpura in a humanized mouse model. *PLoS Pathog* **9**:e1003139.
73. **Bonazzi D, Lo Schiavo V, Machata S, Djafer-Cherif I, Nivoit P, Manriquez V, Tanimoto H, Husson J, Henry N, Chate H, Voituriez R, Dumenil G.** 2018. Intermittent Pili-Mediated Forces Fluidize *Neisseria meningitidis* Aggregates Promoting Vascular Colonization. *Cell* doi:10.1016/j.cell.2018.04.010.
 74. **Mairey E, Genovesio A, Donnadieu E, Bernard C, Jaubert F, Pinard E, Seylaz J, Olivo-Marin JC, Nassif X, Dumenil G.** 2006. Cerebral microcirculation shear stress levels determine *Neisseria meningitidis* attachment sites along the blood-brain barrier. *J Exp Med* **203**:1939-1950.
 75. **Faust SN, Levin M, Harrison OB, Goldin RD, Lockhart MS, Kondaveeti S, Laszik Z, Esmon CT, Heyderman RS.** 2001. Dysfunction of endothelial protein C activation in severe meningococcal sepsis. *N Engl J Med* **345**:408-416.
 76. **Imhaus AF, Dumenil G.** 2014. The number of *Neisseria meningitidis* type IV pili determines host cell interaction. *EMBO J* **33**:1767-1783.
 77. **Bernard SC, Simpson N, Join-Lambert O, Federici C, Laran-Chich MP, Maissa N, Bouzinba-Segard H, Morand PC, Chretien F, Taouji S, Chevet E, Janel S, Lafont F, Coureuil M, Segura A, Niedergang F, Marullo S, Couraud PO, Nassif X, Bourdoulous S.** 2014. Pathogenic *Neisseria meningitidis* utilizes CD147 for vascular colonization. *Nat Med* **20**:725-731.
 78. **Maissa N, Covarelli V, Janel S, Durel B, Simpson N, Bernard SC, Pardo-Lopez L, Bouzinba-Segard H, Faure C, Scott MGH, Coureuil M, Morand PC, Lafont F, Nassif X, Marullo S, Bourdoulous S.** 2017. Strength of *Neisseria meningitidis* binding to endothelial cells requires highly-ordered CD147/beta2-adrenoceptor clusters assembled by alpha-actinin-4. *Nat Commun* **8**:15764.
 79. **Eugene E, Hoffmann I, Pujol C, Couraud PO, Bourdoulous S, Nassif X.** 2002. Microvilli-like structures are associated with the internalization of virulent capsulated *Neisseria meningitidis* into vascular endothelial cells. *J Cell Sci* **115**:1231-1241.
 80. **Soyer M, Charles-Orszag A, Lagache T, Machata S, Imhaus AF, Dumont A, Millien C, Olivo-Marin JC, Dumenil G.** 2014. Early sequence of events triggered by the interaction of *Neisseria meningitidis* with endothelial cells. *Cell Microbiol* **16**:878-895.
 81. **Mikaty G, Soyer M, Mairey E, Henry N, Dyer D, Forest KT, Morand P, Guadagnini S, Prevost MC, Nassif X, Dumenil G.** 2009. Extracellular bacterial pathogen induces host cell surface reorganization to resist shear stress. *PLoS Pathog* **5**:e1000314.
 82. **Merz AJ, So M.** 1997. Attachment of piliated, Opa- and Opc- gonococci and meningococci to epithelial cells elicits cortical actin rearrangements and clustering of tyrosine-phosphorylated proteins. *Infect Immun* **65**:4341-4349.
 83. **Coureuil M, Mikaty G, Miller F, Lecuyer H, Bernard C, Bourdoulous S, Dumenil G, Mege RM, Weksler BB, Romero IA, Couraud PO, Nassif X.** 2009. Meningococcal type IV pili recruit the polarity complex to cross the brain endothelium. *Science* **325**:83-87.
 84. **Hoen B, Duval X.** 2013. Infective endocarditis. *N Engl J Med* **369**:785.
 85. **Cahill TJ, Prendergast BD.** 2016. Infective endocarditis. *Lancet* **387**:882-893.

86. **Vilcant V, Hai O.** 2018. Endocarditis, Bacterial, *StatPearls*, Treasure Island (FL).
87. **McDonald JR, Olaison L, Anderson DJ, Hoen B, Miro JM, Eykyn S, Abrutyn E, Fowler VG, Jr., Habib G, Selton-Suty C, Pappas PA, Cabell CH, Corey GR, Marco F, Sexton DJ.** 2005. Enterococcal endocarditis: 107 cases from the international collaboration on endocarditis merged database. *Am J Med* **118**:759-766.
88. **Yew HS, Murdoch DR.** 2012. Global trends in infective endocarditis epidemiology. *Curr Infect Dis Rep* **14**:367-372.
89. **Widmer E, Que YA, Entenza JM, Moreillon P.** 2006. New concepts in the pathophysiology of infective endocarditis. *Curr Infect Dis Rep* **8**:271-279.
90. **Hemler ME, Elices MJ, Parker C, Takada Y.** 1990. Structure of the integrin VLA-4 and its cell-cell and cell-matrix adhesion functions. *Immunol Rev* **114**:45-65.
91. **Foster TJ, Hook M.** 1998. Surface protein adhesins of *Staphylococcus aureus*. *Trends Microbiol* **6**:484-488.
92. **Chavakis T, Wiechmann K, Preissner KT, Herrmann M.** 2005. *Staphylococcus aureus* interactions with the endothelium: the role of bacterial "secretable expanded repertoire adhesive molecules" (SERAM) in disturbing host defense systems. *Thromb Haemost* **94**:278-285.
93. **Moreillon P, Que YA, Bayer AS.** 2002. Pathogenesis of streptococcal and staphylococcal endocarditis. *Infect Dis Clin North Am* **16**:297-318.
94. **Werdan K, Dietz S, Loffler B, Niemann S, Bushnaq H, Silber RE, Peters G, Muller-Werdan U.** 2014. Mechanisms of infective endocarditis: pathogen-host interaction and risk states. *Nat Rev Cardiol* **11**:35-50.
95. **Haslinger-Loffler B, Kahl BC, Grundmeier M, Strangfeld K, Wagner B, Fischer U, Cheung AL, Peters G, Schulze-Osthoff K, Sinha B.** 2005. Multiple virulence factors are required for *Staphylococcus aureus*-induced apoptosis in endothelial cells. *Cell Microbiol* **7**:1087-1097.
96. **Brouqui P, Raoult D.** 2001. Endocarditis due to rare and fastidious bacteria. *Clin Microbiol Rev* **14**:177-207.
97. **Fournier PE, Lelievre H, Eykyn SJ, Mainardi JL, Marrie TJ, Bruneel F, Roure C, Nash J, Clave D, James E, Benoit-Lemercier C, Deforges L, Tissot-Dupont H, Raoult D.** 2001. Epidemiologic and clinical characteristics of *Bartonella quintana* and *Bartonella henselae* endocarditis: a study of 48 patients. *Medicine (Baltimore)* **80**:245-251.
98. **Raoult D, Fournier PE, Vandenesch F, Mainardi JL, Eykyn SJ, Nash J, James E, Benoit-Lemercier C, Marrie TJ.** 2003. Outcome and treatment of *Bartonella* endocarditis. *Arch Intern Med* **163**:226-230.
99. **Raoult D, Fournier PE, Drancourt M, Marrie TJ, Etienne J, Cosserat J, Cacoub P, Poinsignon Y, Leclercq P, Sefton AM.** 1996. Diagnosis of 22 new cases of *Bartonella* endocarditis. *Ann Intern Med* **125**:646-652.
100. **Dehio C.** 2004. Molecular and cellular basis of *bartonella* pathogenesis. *Annu Rev Microbiol* **58**:365-390.
101. **Breitschwerdt EB, Kordick DL.** 2000. *Bartonella* infection in animals: carriership, reservoir potential, pathogenicity, and zoonotic potential for human infection. *Clin Microbiol Rev* **13**:428-438.
102. **Chomel BB.** 2000. Cat-scratch disease. *Rev Sci Tech* **19**:136-150.

103. **Koehler JE, Tappero JW.** 1993. Bacillary angiomatosis and bacillary peliosis in patients infected with human immunodeficiency virus. *Clin Infect Dis* **17**:612-624.
104. **Regnery RL, Childs JE, Koehler JE.** 1995. Infections associated with *Bartonella* species in persons infected with human immunodeficiency virus. *Clin Infect Dis* **21 Suppl 1**:S94-98.
105. **Kostianovsky M, Greco MA.** 1994. Angiogenic process in bacillary angiomatosis. *Ultrastruct Pathol* **18**:349-355.
106. **Manders SM.** 1996. Bacillary angiomatosis. *Clin Dermatol* **14**:295-299.
107. **Dehio C, Meyer M, Berger J, Schwarz H, Lanz C.** 1997. Interaction of *Bartonella henselae* with endothelial cells results in bacterial aggregation on the cell surface and the subsequent engulfment and internalisation of the bacterial aggregate by a unique structure, the invasome. *J Cell Sci* **110 (Pt 18)**:2141-2154.
108. **Verma A, Davis GE, Ihler GM.** 2000. Infection of human endothelial cells with *Bartonella bacilliformis* is dependent on Rho and results in activation of Rho. *Infect Immun* **68**:5960-5969.
109. **Dramsi S, Cossart P.** 1998. Intracellular pathogens and the actin cytoskeleton. *Annu Rev Cell Dev Biol* **14**:137-166.
110. **Kirby JE, Nekorchuk DM.** 2002. *Bartonella*-associated endothelial proliferation depends on inhibition of apoptosis. *Proc Natl Acad Sci U S A* **99**:4656-4661.
111. **Schulein R, Dehio C.** 2002. The VirB/VirD4 type IV secretion system of *Bartonella* is essential for establishing intraerythrocytic infection. *Mol Microbiol* **46**:1053-1067.
112. **Seubert A, Hiestand R, de la Cruz F, Dehio C.** 2003. A bacterial conjugation machinery recruited for pathogenesis. *Mol Microbiol* **49**:1253-1266.
113. **Torisu H, Ono M, Kiryu H, Furue M, Ohmoto Y, Nakayama J, Nishioka Y, Sone S, Kuwano M.** 2000. Macrophage infiltration correlates with tumor stage and angiogenesis in human malignant melanoma: possible involvement of TNFalpha and IL-1alpha. *Int J Cancer* **85**:182-188.
114. **Musso T, Badolato R, Ravarino D, Stornello S, Panzanelli P, Merlino C, Savoia D, Cavallo R, Ponzi AN, Zucca M.** 2001. Interaction of *Bartonella henselae* with the murine macrophage cell line J774: infection and proinflammatory response. *Infect Immun* **69**:5974-5980.
115. **Kempf VA, Volkmann B, Schaller M, Sander CA, Alitalo K, Riess T, Autenrieth IB.** 2001. Evidence of a leading role for VEGF in *Bartonella henselae*-induced endothelial cell proliferations. *Cell Microbiol* **3**:623-632.
116. **Resto-Ruiz SI, Schmiederer M, Sweger D, Newton C, Klein TW, Friedman H, Anderson BE.** 2002. Induction of a potential paracrine angiogenic loop between human THP-1 macrophages and human microvascular endothelial cells during *Bartonella henselae* infection. *Infect Immun* **70**:4564-4570.
117. **Minasyan H.** 2016. Mechanisms and pathways for the clearance of bacteria from blood circulation in health and disease. *Pathophysiology* **23**:61-66.
118. **Khakpour S, Wilhelmsen K, Hellman J.** 2015. Vascular endothelial cell Toll-like receptor pathways in sepsis. *Innate Immun* **21**:827-846.
119. **Gupta SK, Lysko PG, Pillarisetti K, Ohlstein E, Stadel JM.** 1998. Chemokine receptors in human endothelial cells. Functional expression of CXCR4 and its transcriptional regulation by inflammatory cytokines. *J Biol Chem* **273**:4282-4287.

120. **Murdoch C, Monk PN, Finn A.** 1999. Cxc chemokine receptor expression on human endothelial cells. *Cytokine* **11**:704-712.
121. **Mitchell JA, Ryffel B, Quesniaux VF, Cartwright N, Paul-Clark M.** 2007. Role of pattern-recognition receptors in cardiovascular health and disease. *Biochem Soc Trans* **35**:1449-1452.
122. **Opitz B, Eitel J, Meixenberger K, Suttorp N.** 2009. Role of Toll-like receptors, NOD-like receptors and RIG-I-like receptors in endothelial cells and systemic infections. *Thromb Haemost* **102**:1103-1109.
123. **Marceau F, Grassi J, Frobert Y, Bergeron C, Poubelle PE.** 1992. Effects of experimental conditions on the production of interleukin-1 alpha and -1 beta by human endothelial cells cultured in vitro. *Int J Immunopharmacol* **14**:525-534.
124. **Opitz B, Puschel A, Beermann W, Hocke AC, Forster S, Schmeck B, van Laak V, Chakraborty T, Suttorp N, Hippenstiel S.** 2006. *Listeria monocytogenes* activated p38 MAPK and induced IL-8 secretion in a nucleotide-binding oligomerization domain 1-dependent manner in endothelial cells. *J Immunol* **176**:484-490.
125. **Anand AR, Cucchiaroni M, Terwilliger EF, Ganju RK.** 2008. The tyrosine kinase Pyk2 mediates lipopolysaccharide-induced IL-8 expression in human endothelial cells. *J Immunol* **180**:5636-5644.
126. **Faure E, Thomas L, Xu H, Medvedev A, Equils O, Arditi M.** 2001. Bacterial lipopolysaccharide and IFN-gamma induce Toll-like receptor 2 and Toll-like receptor 4 expression in human endothelial cells: role of NF-kappa B activation. *J Immunol* **166**:2018-2024.
127. **Danese S, Dejana E, Fiocchi C.** 2007. Immune regulation by microvascular endothelial cells: directing innate and adaptive immunity, coagulation, and inflammation. *J Immunol* **178**:6017-6022.
128. **Neefjes J, Jongsma ML, Paul P, Bakke O.** 2011. Towards a systems understanding of MHC class I and MHC class II antigen presentation. *Nat Rev Immunol* **11**:823-836.
129. **Rose ML, Coles MI, Griffin RJ, Pomerance A, Yacoub MH.** 1986. Expression of class I and class II major histocompatibility antigens in normal and transplanted human heart. *Transplantation* **41**:776-780.
130. **Leeuwenberg JF, Van Damme J, Meager T, Jeunhomme TM, Buurman WA.** 1988. Effects of tumor necrosis factor on the interferon-gamma-induced major histocompatibility complex class II antigen expression by human endothelial cells. *Eur J Immunol* **18**:1469-1472.
131. **Mai J, Virtue A, Shen J, Wang H, Yang XF.** 2013. An evolving new paradigm: endothelial cells--conditional innate immune cells. *J Hematol Oncol* **6**:61.
132. **van der Poll T, van de Veerdonk FL, Scicluna BP, Netea MG.** 2017. The immunopathology of sepsis and potential therapeutic targets. *Nat Rev Immunol* **17**:407-420.
133. **Wiersinga WJ, Leopold SJ, Cranendonk DR, van der Poll T.** 2014. Host innate immune responses to sepsis. *Virulence* **5**:36-44.
134. **Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G, Sccm/Esicm/Accp/Ats/Sis.** 2003. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med* **31**:1250-1256.
135. **Rittirsch D, Flierl MA, Ward PA.** 2008. Harmful molecular mechanisms in sepsis. *Nat Rev Immunol* **8**:776-787.

136. Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent JL. 2016. Sepsis and septic shock. *Nat Rev Dis Primers* **2**:16045.
137. Hotchkiss RS, Tinsley KW, Swanson PE, Grayson MH, Osborne DF, Wagner TH, Cobb JP, Coopersmith C, Karl IE. 2002. Depletion of dendritic cells, but not macrophages, in patients with sepsis. *J Immunol* **168**:2493-2500.
138. Hotchkiss RS, Monneret G, Payen D. 2013. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat Rev Immunol* **13**:862-874.
139. Scumpia PO, Delano MJ, Kelly-Scumpia KM, Weinstein JS, Wynn JL, Winfield RD, Xia C, Chung CS, Ayala A, Atkinson MA, Reeves WH, Clare-Salzler MJ, Moldawer LL. 2007. Treatment with G1TR agonistic antibody corrects adaptive immune dysfunction in sepsis. *Blood* **110**:3673-3681.
140. Boomer JS, To K, Chang KC, Takasu O, Osborne DF, Walton AH, Bricker TL, Jarman SD, 2nd, Kreisel D, Krupnick AS, Srivastava A, Swanson PE, Green JM, Hotchkiss RS. 2011. Immunosuppression in patients who die of sepsis and multiple organ failure. *JAMA* **306**:2594-2605.
141. Huang X, Venet F, Wang YL, Lepape A, Yuan Z, Chen Y, Swan R, Kherouf H, Monneret G, Chung CS, Ayala A. 2009. PD-1 expression by macrophages plays a pathologic role in altering microbial clearance and the innate inflammatory response to sepsis. *Proc Natl Acad Sci U S A* **106**:6303-6308.
142. Levi M, Ten Cate H. 1999. Disseminated intravascular coagulation. *N Engl J Med* **341**:586-592.
143. Davis RP, Miller-Dorey S, Jenne CN. 2016. Platelets and coagulation in infection. *Clin Transl Immunology* **5**:e89.
144. Pernerstorfer T, Stohlawetz P, Hollenstein U, Dzirlo L, Eichler HG, Kapiotis S, Jilma B, Speiser W. 1999. Endotoxin-induced activation of the coagulation cascade in humans: effect of acetylsalicylic acid and acetaminophen. *Arterioscler Thromb Vasc Biol* **19**:2517-2523.
145. Osterud B, Bjorklid E. 2001. The tissue factor pathway in disseminated intravascular coagulation. *Semin Thromb Hemost* **27**:605-617.
146. Abraham E. 2000. Coagulation abnormalities in acute lung injury and sepsis. *Am J Respir Cell Mol Biol* **22**:401-404.
147. Ince C. 2005. The microcirculation is the motor of sepsis. *Crit Care* **9 Suppl 4**:S13-19.

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.

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

854

855 **Figure 3: Vascular colonization by *Neisseria meningitidis*.** Once into the

856 bloodstream, *Neisseria meningitidis* adheres to the endothelium thanks to the surface

857 expression of type four pili (Tfp). While proliferating, and owing to their auto-

858 aggregative property, bacteria form a tight microcolony at the surface of the

859 endothelium, which ultimately leads to the congestion of the colonized vessel.

860 Bacterial adhesion at the surface of endothelial cells induces a drastic remodeling of

861 the host cell-plasma membrane that forms membrane protrusions interdigitating

862 within the bacterial aggregate. In addition, pilus interaction with endothelial cell-

863 surface receptors, such as CD147 or β 2-adrenergic receptor (β 2-AR), induces the

864 reorganization of the actin cytoskeleton and intercellular junctions by recruiting their

865 components underneath the microcolony. Taken together, these events are proposed

866 to destabilize intercellular junctions, hence resulting in the increase in vessel

867 permeability.

868

869

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.

878

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 intravesicular 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.