



**HAL**  
open science

# The Many Faces of Bacterium-Endothelium Interactions during Systemic Infections

Dorian Obino, Guillaume Duménil

► **To cite this version:**

Dorian Obino, Guillaume Duménil. The Many Faces of Bacterium-Endothelium Interactions during Systemic Infections. Pascale Cossart; Craig R. Roy; Philippe Sansonetti. Bacteria and Intracellularly, John Wiley & Sons, Inc., pp.69-81, 2020, 9781683670254 (print); 9781683670261 (online). 10.1128/9781683670261.ch5 . pasteur-03116237

**HAL Id: pasteur-03116237**

**<https://pasteur.hal.science/pasteur-03116237>**

Submitted on 20 Jan 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License



## 23 THE ENDOTHELIUM AS A SITE OF BACTERIAL INFECTIONS

24 The endothelium refers to the layer of endothelial cells (ECs) lining the inner surface  
25 of blood vessels that span the entire body and ensure the distribution of blood  
26 throughout the organism (1). It can be estimated that the human body contains the  
27 staggering number of 100 000 km of blood vessels, more than twice the earth's  
28 circumference (2). Therefore, a bacterium reaching the circulation is engaged in a  
29 maze of huge proportion. Moreover, a pathogen travelling throughout the circulation  
30 does not encounter a homogeneous environment, as an important feature of the  
31 vascular network is its diversity. Although endothelial cells are present in all vessels,  
32 the organization of the vessel wall – formed by three layers referred to as the *tunica*  
33 *intima*, *media* and *adventia* (from the vessel lumen outward) – is different among  
34 different vessel types and different organs (3). Vessels can be first differentiated by  
35 the complex extracellular matrix layers surrounding them. For instance, elastic  
36 arteries such as the aorta are surrounded by 50 elastic layers providing them unique  
37 mechanical properties (3). Second, the cellular content is also different according to  
38 vessel type, the wall of arteries and veins contains a layer of smooth muscle cells  
39 (SMCs) that provides their capacity to relax or constrict in response to vasoactive  
40 molecules (4). An additional level of complexity in the network stems from the fact  
41 that larger vessels, veins or arteries are themselves vascularized by smaller vessels,  
42 the *vasa vasorum* (5). Although endothelial cells are constituent of all vessels, they  
43 themselves present different properties depending on their anatomical location, in  
44 particular in the case of capillaries. The lumen of *continuous capillaries*, which are  
45 the most common, are lined with an uninterrupted layer of endothelial cells.  
46 *Fenestrated capillaries*, typically present in glomeruli of the kidney, are laced with 50-  
47 80 nm openings thus changing their permeability properties. In the liver, *sinusoidal*

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

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

71

## 72 **CELLULAR JUNCTIONS AS THE GATEKEEPERS OF THE ENDOTHELIAL** 73 **BARRIER**

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

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

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

120

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

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

127

## 128 **ALTERATION OF VASCULAR INTEGRITY UPON INFECTIONS BY *RICKETTSIA***

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

142 Adhesion of *Rickettsia* onto endothelial cells also leads to a drastic remodeling of the  
143 actin cytoskeleton within the host cells that not only facilitates bacterial entry but also  
144 participates to bacteria movement and spreading within the endothelium. Endothelial  
145 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- $\kappa$ 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).



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

179

## 180 **VASCULAR COLONIZATION AND BLOOD-BRAIN BARRIER CROSSING BY** 181 ***NEISSERIA MENINGITIDIS***

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

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

195 while neonatal meningitis is mainly triggered by group B *Streptococcus*, *Listeria*  
196 *monocytogenes* and *Escherichia coli* K1 (68), immunocompromised patients  
197 frequently developed *Mycobacterium tuberculosis* or non-typhoid *Salmonella*  
198 meningitis (69, 70). Despite their heterogeneity, common themes in the mechanisms  
199 used by these pathogenic bacteria to cross the BBB can be drawn (for review, see  
200 (71)).

201 Recent studies have pointed to the importance of a process called *vascular*  
202 *colonization* during *N. meningitidis* infections and probably during meningitis (72).  
203 This bacterium has the ability to adhere to the endothelial surface and proliferate in  
204 the form of bacterial aggregates that eventually fill the lumen of small vessels of 10 to  
205 50  $\mu\text{m}$  in diameter (Figure 3). A recent study shows that this colonization process is  
206 facilitated by the honey-like viscous liquid properties of the bacterial aggregates  
207 which allow them to adapt to the complex morphology of the vasculature upon  
208 proliferation (73). Adhesion to the endothelium likely participates in the immune  
209 evasion as this prevents phagocytosis from K upffer cells in the liver. Histological  
210 analysis of *post-mortem* samples reveals large bacterial aggregates in the brain  
211 vessels suggesting that vascular colonization could promote BBB crossing by  
212 concentrating a high number of bacteria at specific sites and by altering endothelial  
213 cell physiology (74). The combined effect of vessel occlusion due to bacterial  
214 accumulation and the activation of the coagulation cascade (75) might participate in  
215 altering endothelium integrity.

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

220 CD147 and  $\beta$ 2-adrenergic receptor (77, 78). While proliferating at the surface of  
221 infected endothelial cells, thus forming bacterial aggregates, meningococci induce  
222 the remodeling of the host cell plasma membrane (79, 80) (Figure 3). Associated to  
223 their aggregation capacity, plasma membrane protrusions infiltrating meningococcal  
224 microcolonies were shown to enhance the mechanical cohesion of the microcolony  
225 thus allowing them to resist the blood-flow induced shear stress (81). Interestingly,  
226 although the cortical actin network is strongly reorganized below the bacterial  
227 colonies (82), the active contribution of the host cells has been shown to be  
228 dispensable in the *Nm*-mediated plasma membrane remodeling. The actin  
229 cytoskeleton or even the intracellular ATP is not necessary for the Tfp-induced  
230 plasma membrane reorganization (80, 81). Upon bacterial adhesion to microvascular  
231 endothelial cells, Tfp trigger a complex cascade of signaling events leading to the  
232 formation of “ectopic junctions” underneath bacterial aggregates together with the  
233 local remodeling of the cortical actin cytoskeleton (83). This process relies, on the  
234 one hand, on the activation of small GTPases such as cell division cycle protein 42  
235 (Cdc42) and Rac1, along with the local recruitment of proteins of the polarity  
236 complex, including partitioning-defective 3 (PAR3), PAR6 and protein kinase C- $\zeta$   
237 (PKC- $\zeta$ ), as well as the branched-actin nucleating complex Arp2/3. On the other  
238 hand, by mistargeting recycling endosomes, meningococci induce the accumulation  
239 of junctional proteins, such as VE-Cadherin, underneath bacterial aggregates, a  
240 process shown to weaken intercellular junctions and increase blood-brain barrier  
241 permeability, thus facilitating meningococcal dissemination within the cerebral tissues  
242 and the cerebrospinal fluid (CSF) (83). These elaborated mechanisms illustrate the  
243 panoply of strategies enabling pathogenic bacteria to alter the endothelial barrier.

244

## 245 **ALTERATION OF THE ENDOCARDIUM AND ENDOCARDITIS**

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

252 The pathologic cascade starts following sterile lesions of the cardiac valve  
253 endothelium of unclear origin that lead to the exposure of the extracellular matrix.  
254 This triggers the formation of a platelet- and fibrin-rich thrombus, considered as a  
255 hot-spot for the adhesion of blood-circulating bacteria (89) (Figure 4). Alternatively,  
256 bacterial adhesion can occur at the surface of inflamed endothelium, a process  
257 facilitated by the local upregulation of cell surface adhesion molecules, such as  $\beta$ 1  
258 integrins (90). From the bacterial side, adhesion is mediated by the surface  
259 expression of extracellular matrix-targeting adhesins, such as fibronectin binding  
260 proteins (FnBPs) (91, 92). Adherent bacteria locally proliferate and form a *vegetation*,  
261 a biofilm-like structure where aggregated bacteria are mixed together with  
262 extracellular matrix proteins, clot components and/or immune cells (93). While the  
263 vegetation matures, the adjacent endothelial cells are exposed, thus driving the  
264 propagation of the local inflammation and cell death and ultimately leading to the  
265 destruction of the infected valves thus requiring surgical replacement (94). However,  
266 this mechanism probably does not entirely account for IE induced by intracellular  
267 bacteria such as *Bartonella* species or *Staphylococcus aureus*, which rather rely on  
268 the secretion of exoenzymes and toxins to mediate their pathogenic effects (95) and  
269 for which the host immune response might play an important role (96). Although

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

273

#### 274 **ANGIOPROLIFERATION DURING *BARTONELLA* INFECTIONS**

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

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

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

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

309

## 310 **SYSTEMIC IMPLICATIONS OF VASCULAR INFECTIONS**

311 Under steady-state conditions, besides normal transitory bacteremia, the vascular  
312 organ is thought to be sterile (26). Therefore, it possesses robust mechanisms to  
313 recognize circulating pathogens and trigger innate and/or adaptive immune  
314 responses (117, 118). Similarly to immune cells specialized in pathogen recognition,  
315 endothelial cells express chemokine receptors, such as CXCR-1, -2 and -4 (119,  
316 120) as well as pattern-recognition receptors (PRRs), such as Toll-Like Receptors  
317 (TLRs) and NOD-Like Receptors (NLRs) (121, 122). They have also been shown to  
318 secrete pro-inflammatory molecules, such as the cytokines interleukin (IL)-1 and -8  
319 (123-125) and to respond to bacterial lipopolysaccharide (LPS), tumor necrosis

320 factor- $\alpha$  (TNF- $\alpha$ ) or interferon- $\gamma$  (INF- $\gamma$ ) by signaling through the canonical pro-  
321 inflammatory NF- $\kappa$ 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- $\kappa$ 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



369 the stability of intercellular junctions and the integrity of the vascular endothelium  
370 (118). As a consequence, vessels become leaky, blood pressure decreases and  
371 proteins from the endothelial extracellular matrix, such as collagen, are exposed to  
372 the vessel content, which further activates platelet aggregation and fibrin formation  
373 (118, 143). Multi-organ failure is often associated with the late phases of DIC, which  
374 results from microvascular thrombosis and poor tissue perfusion (146, 147).

375

### 376 **CONCLUDING REMARKS**

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

390

391

392

393

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.

400 **References**

- 401 1. **Lerman A, Burnett JC, Jr.** 1992. Intact and altered endothelium in regulation  
402 of vasomotion. *Circulation* **86**:III12-19.
- 403 2. **Sender R, Fuchs S, Milo R.** 2016. Revised Estimates for the Number of  
404 Human and Bacteria Cells in the Body. *PLoS Biol* **14**:e1002533.
- 405 3. **Pugsley MK, Tabrizchi R.** 2000. The vascular system. An overview of  
406 structure and function. *J Pharmacol Toxicol Methods* **44**:333-340.
- 407 4. **Brozovich FV, Nicholson CJ, Degen CV, Gao YZ, Aggarwal M, Morgan**  
408 **KG.** 2016. Mechanisms of Vascular Smooth Muscle Contraction and the Basis  
409 for Pharmacologic Treatment of Smooth Muscle Disorders. *Pharmacol Rev*  
410 **68**:476-532.
- 411 5. **Galili O, Herrmann J, Woodrum J, Sattler KJ, Lerman LO, Lerman A.**  
412 2004. Adventitial vasa vasorum heterogeneity among different vascular beds.  
413 *J Vasc Surg* **40**:529-535.
- 414 6. **Sarin H.** 2010. Physiologic upper limits of pore size of different blood capillary  
415 types and another perspective on the dual pore theory of microvascular  
416 permeability. *J Angiogenes Res* **2**:14.
- 417 7. **Bilzer M, Roggel F, Gerbes AL.** 2006. Role of Kupffer cells in host defense  
418 and liver disease. *Liver Int* **26**:1175-1186.
- 419 8. **Harris IS, Black BL.** 2010. Development of the endocardium. *Pediatr Cardiol*  
420 **31**:391-399.
- 421 9. **Carmeliet P.** 2003. Angiogenesis in health and disease. *Nat Med* **9**:653-660.
- 422 10. **Zecchin A, Kalucka J, Dubois C, Carmeliet P.** 2017. How Endothelial Cells  
423 Adapt Their Metabolism to Form Vessels in Tumors. *Front Immunol* **8**:1750.
- 424 11. **Risau W, Flamme I.** 1995. Vasculogenesis. *Annu Rev Cell Dev Biol* **11**:73-91.
- 425 12. **Risau W.** 1997. Mechanisms of angiogenesis. *Nature* **386**:671-674.
- 426 13. **Diaz-Santana A, Shan M, Stroock AD.** 2015. Endothelial cell dynamics  
427 during anastomosis in vitro. *Integr Biol (Camb)* **7**:454-466.
- 428 14. **Komarova Y, Malik AB.** 2010. Regulation of endothelial permeability via  
429 paracellular and transcellular transport pathways. *Annu Rev Physiol* **72**:463-  
430 493.
- 431 15. **Hawkins BT, Davis TP.** 2005. The blood-brain barrier/neurovascular unit in  
432 health and disease. *Pharmacol Rev* **57**:173-185.
- 433 16. **Daneman R, Prat A.** 2015. The blood-brain barrier. *Cold Spring Harb*  
434 *Perspect Biol* **7**:a020412.
- 435 17. **Rudini N, Dejana E.** 2008. Adherens junctions. *Curr Biol* **18**:R1080-1082.
- 436 18. **Privratsky JR, Newman PJ.** 2014. PECAM-1: regulator of endothelial  
437 junctional integrity. *Cell Tissue Res* **355**:607-619.
- 438 19. **Hartsock A, Nelson WJ.** 2008. Adherens and tight junctions: structure,  
439 function and connections to the actin cytoskeleton. *Biochim Biophys Acta*  
440 **1778**:660-669.
- 441 20. **Bates DO, Harper SJ.** 2002. Regulation of vascular permeability by vascular  
442 endothelial growth factors. *Vascul Pharmacol* **39**:225-237.
- 443 21. **Bates DO.** 2010. Vascular endothelial growth factors and vascular  
444 permeability. *Cardiovasc Res* **87**:262-271.
- 445 22. **Wu HM, Huang Q, Yuan Y, Granger HJ.** 1996. VEGF induces NO-dependent  
446 hyperpermeability in coronary venules. *Am J Physiol* **271**:H2735-2739.

- 447 23. **Aghajanian A, Wittchen ES, Allingham MJ, Garrett TA, Burridge K.** 2008.  
448 Endothelial cell junctions and the regulation of vascular permeability and  
449 leukocyte transmigration. *J Thromb Haemost* **6**:1453-1460.
- 450 24. **Popoff MR, Geny B.** 2009. Multifaceted role of Rho, Rac, Cdc42 and Ras in  
451 intercellular junctions, lessons from toxins. *Biochim Biophys Acta* **1788**:797-  
452 812.
- 453 25. **Round H, Kirkpatrick HJ, Hails CG.** 1936. Further Investigations on  
454 Bacteriological Infections of the Mouth: (Section of Odontology). *Proc R Soc*  
455 *Med* **29**:1552-1556.
- 456 26. **Cobe HM.** 1954. Transitory bacteremia. *Oral Surg Oral Med Oral Pathol*  
457 **7**:609-615.
- 458 27. **Snowden J, Bhimji SS.** 2018. Rickettsial Infection, *StatPearls*, Treasure  
459 Island (FL).
- 460 28. **Stins MF, Prasadarao NV, Ibric L, Wass CA, Luckett P, Kim KS.** 1994.  
461 Binding characteristics of S fimbriated Escherichia coli to isolated brain  
462 microvascular endothelial cells. *Am J Pathol* **145**:1228-1236.
- 463 29. **Greiffenberg L, Goebel W, Kim KS, Weiglein I, Bubert A, Engelbrecht F,**  
464 **Stins M, Kuhn M.** 1998. Interaction of *Listeria monocytogenes* with human  
465 brain microvascular endothelial cells: InlB-dependent invasion, long-term  
466 intracellular growth, and spread from macrophages to endothelial cells. *Infect*  
467 *Immun* **66**:5260-5267.
- 468 30. **Sheen TR, Ebrahimi CM, Hiemstra IH, Barlow SB, Peschel A, Doran KS.**  
469 2010. Penetration of the blood-brain barrier by *Staphylococcus aureus*:  
470 contribution of membrane-anchored lipoteichoic acid. *J Mol Med (Berl)*  
471 **88**:633-639.
- 472 31. **Stins MF, Badger J, Sik Kim K.** 2001. Bacterial invasion and transcytosis in  
473 transfected human brain microvascular endothelial cells. *Microb Pathog*  
474 **30**:19-28.
- 475 32. **Jain SK, Paul-Satyaseela M, Lamichhane G, Kim KS, Bishai WR.** 2006.  
476 Mycobacterium tuberculosis invasion and traversal across an in vitro human  
477 blood-brain barrier as a pathogenic mechanism for central nervous system  
478 tuberculosis. *J Infect Dis* **193**:1287-1295.
- 479 33. **Nikulin J, Panzner U, Frosch M, Schubert-Unkmeir A.** 2006. Intracellular  
480 survival and replication of *Neisseria meningitidis* in human brain microvascular  
481 endothelial cells. *Int J Med Microbiol* **296**:553-558.
- 482 34. **Prasadarao NV, Wass CA, Stins MF, Shimada H, Kim KS.** 1999. Outer  
483 membrane protein A-promoted actin condensation of brain microvascular  
484 endothelial cells is required for *Escherichia coli* invasion. *Infect Immun*  
485 **67**:5775-5783.
- 486 35. **Das A, Asatryan L, Reddy MA, Wass CA, Stins MF, Joshi S, Bonventre**  
487 **JV, Kim KS.** 2001. Differential role of cytosolic phospholipase A2 in the  
488 invasion of brain microvascular endothelial cells by *Escherichia coli* and  
489 *Listeria monocytogenes*. *J Infect Dis* **184**:732-737.
- 490 36. **Dumenil G.** 2011. Revisiting the extracellular lifestyle. *Cell Microbiol* **13**:1114-  
491 1121.
- 492 37. **Olano JP.** 2005. Rickettsial infections. *Ann N Y Acad Sci* **1063**:187-196.
- 493 38. **Walker DH, Ismail N.** 2008. Emerging and re-emerging rickettsioses:  
494 endothelial cell infection and early disease events. *Nat Rev Microbiol* **6**:375-  
495 386.

- 496 39. **Li H, Walker DH.** 1998. rOmpA is a critical protein for the adhesion of  
497 Rickettsia rickettsii to host cells. *Microb Pathog* **24**:289-298.
- 498 40. **Hillman RD, Jr., Baktash YM, Martinez JJ.** 2013. OmpA-mediated rickettsial  
499 adherence to and invasion of human endothelial cells is dependent upon  
500 interaction with alpha2beta1 integrin. *Cell Microbiol* **15**:727-741.
- 501 41. **Walker TS, Winkler HH.** 1978. Penetration of cultured mouse fibroblasts (L  
502 cells) by Rickettsia prowazeki. *Infect Immun* **22**:200-208.
- 503 42. **Walker TS.** 1984. Rickettsial interactions with human endothelial cells in vitro:  
504 adherence and entry. *Infect Immun* **44**:205-210.
- 505 43. **Chan YG, Cardwell MM, Hermanas TM, Uchiyama T, Martinez JJ.** 2009.  
506 Rickettsial outer-membrane protein B (rOmpB) mediates bacterial invasion  
507 through Ku70 in an actin, c-Cbl, clathrin and caveolin 2-dependent manner.  
508 *Cell Microbiol* **11**:629-644.
- 509 44. **Martinez JJ, Cossart P.** 2004. Early signaling events involved in the entry of  
510 Rickettsia conorii into mammalian cells. *J Cell Sci* **117**:5097-5106.
- 511 45. **Radulovic S, Troyer JM, Beier MS, Lau AO, Azad AF.** 1999. Identification  
512 and molecular analysis of the gene encoding Rickettsia typhi hemolysin. *Infect*  
513 *Immun* **67**:6104-6108.
- 514 46. **Renesto P, Dehoux P, Gouin E, Touqui L, Cossart P, Raoult D.** 2003.  
515 Identification and characterization of a phospholipase D-superfamily gene in  
516 rickettsiae. *J Infect Dis* **188**:1276-1283.
- 517 47. **Whitworth T, Popov VL, Yu XJ, Walker DH, Bouyer DH.** 2005. Expression  
518 of the Rickettsia prowazekii pld or tlyC gene in Salmonella enterica serovar  
519 Typhimurium mediates phagosomal escape. *Infect Immun* **73**:6668-6673.
- 520 48. **Walker DH, Valbuena GA, Olano JP.** 2003. Pathogenic mechanisms of  
521 diseases caused by Rickettsia. *Ann N Y Acad Sci* **990**:1-11.
- 522 49. **Heinzen RA.** 2003. Rickettsial actin-based motility: behavior and involvement  
523 of cytoskeletal regulators. *Ann N Y Acad Sci* **990**:535-547.
- 524 50. **Jeng RL, Goley ED, D'Alessio JA, Chaga OY, Svitkina TM, Borisy GG,  
525 Heinzen RA, Welch MD.** 2004. A Rickettsia WASP-like protein activates the  
526 Arp2/3 complex and mediates actin-based motility. *Cell Microbiol* **6**:761-769.
- 527 51. **Gouin E, Egile C, Dehoux P, Villiers V, Adams J, Gertler F, Li R, Cossart  
528 P.** 2004. The RickA protein of Rickettsia conorii activates the Arp2/3 complex.  
529 *Nature* **427**:457-461.
- 530 52. **Gouin E, Gantelet H, Egile C, Lasa I, Ohayon H, Villiers V, Gounon P,  
531 Sansonetti PJ, Cossart P.** 1999. A comparative study of the actin-based  
532 motilities of the pathogenic bacteria Listeria monocytogenes, Shigella flexneri  
533 and Rickettsia conorii. *J Cell Sci* **112 ( Pt 11)**:1697-1708.
- 534 53. **Harlander RS, Way M, Ren Q, Howe D, Grieshaber SS, Heinzen RA.** 2003.  
535 Effects of ectopically expressed neuronal Wiskott-Aldrich syndrome protein  
536 domains on Rickettsia rickettsii actin-based motility. *Infect Immun* **71**:1551-  
537 1556.
- 538 54. **Haglund CM, Choe JE, Skau CT, Kovar DR, Welch MD.** 2010. Rickettsia  
539 Sca2 is a bacterial formin-like mediator of actin-based motility. *Nat Cell Biol*  
540 **12**:1057-1063.
- 541 55. **Kleba B, Clark TR, Lutter EI, Ellison DW, Hackstadt T.** 2010. Disruption of  
542 the Rickettsia rickettsii Sca2 autotransporter inhibits actin-based motility. *Infect*  
543 *Immun* **78**:2240-2247.
- 544 56. **Sahni SK, Narra HP, Sahni A, Walker DH.** 2013. Recent molecular insights  
545 into rickettsial pathogenesis and immunity. *Future Microbiol* **8**:1265-1288.

- 546 57. **Sporn LA, Sahni SK, Lerner NB, Marder VJ, Silverman DJ, Turpin LC,**  
547 **Schwab AL.** 1997. Rickettsia rickettsii infection of cultured human endothelial  
548 cells induces NF-kappaB activation. *Infect Immun* **65**:2786-2791.
- 549 58. **Sahni SK, Van Antwerp DJ, Ereemeeva ME, Silverman DJ, Marder VJ,**  
550 **Sporn LA.** 1998. Proteasome-independent activation of nuclear factor kappaB  
551 in cytoplasmic extracts from human endothelial cells by Rickettsia rickettsii.  
552 *Infect Immun* **66**:1827-1833.
- 553 59. **Clifton DR, Goss RA, Sahni SK, van Antwerp D, Baggs RB, Marder VJ,**  
554 **Silverman DJ, Sporn LA.** 1998. NF-kappa B-dependent inhibition of  
555 apoptosis is essential for host cell survival during Rickettsia rickettsii infection.  
556 *Proc Natl Acad Sci U S A* **95**:4646-4651.
- 557 60. **Walker DH.** 2007. Rickettsiae and rickettsial infections: the current state of  
558 knowledge. *Clin Infect Dis* **45 Suppl 1**:S39-44.
- 559 61. **Walker DH, Olano JP, Feng HM.** 2001. Critical role of cytotoxic T  
560 lymphocytes in immune clearance of rickettsial infection. *Infect Immun*  
561 **69**:1841-1846.
- 562 62. **Valbuena G, Walker DH.** 2005. Changes in the adherens junctions of human  
563 endothelial cells infected with spotted fever group rickettsiae. *Virchows Arch*  
564 **446**:379-382.
- 565 63. **Santucci LA, Gutierrez PL, Silverman DJ.** 1992. Rickettsia rickettsii induces  
566 superoxide radical and superoxide dismutase in human endothelial cells.  
567 *Infect Immun* **60**:5113-5118.
- 568 64. **Ereemeeva ME, Silverman DJ.** 1998. Effects of the antioxidant alpha-lipoic  
569 acid on human umbilical vein endothelial cells infected with Rickettsia  
570 rickettsii. *Infect Immun* **66**:2290-2299.
- 571 65. **Schmaier AH, Srikanth S, Elghetany MT, Normolle D, Gokhale S, Feng**  
572 **HM, Walker DH.** 2001. Hemostatic/fibrinolytic protein changes in C3H/HeN  
573 mice infected with Rickettsia conorii--a model for Rocky Mountain spotted  
574 fever. *Thromb Haemost* **86**:871-879.
- 575 66. **Doran KS, Fulde M, Gratz N, Kim BJ, Nau R, Prasadarao N, Schubert-**  
576 **Unkmeir A, Tuomanen EI, Valentin-Weigand P.** 2016. Host-pathogen  
577 interactions in bacterial meningitis. *Acta Neuropathol* **131**:185-209.
- 578 67. **Brandtzaeg P, van Deuren M.** 2012. Classification and pathogenesis of  
579 meningococcal infections. *Methods Mol Biol* **799**:21-35.
- 580 68. **Thigpen MC, Whitney CG, Messonnier NE, Zell ER, Lynfield R, Hadler JL,**  
581 **Harrison LH, Farley MM, Reingold A, Bennett NM, Craig AS, Schaffner W,**  
582 **Thomas A, Lewis MM, Scallan E, Schuchat A, Emerging Infections**  
583 **Programs N.** 2011. Bacterial meningitis in the United States, 1998-2007. *N*  
584 *Engl J Med* **364**:2016-2025.
- 585 69. **Bekondi C, Bernede C, Passone N, Minssart P, Kamalo C, Mbolidi D,**  
586 **Germani Y.** 2006. Primary and opportunistic pathogens associated with  
587 meningitis in adults in Bangui, Central African Republic, in relation to human  
588 immunodeficiency virus serostatus. *Int J Infect Dis* **10**:387-395.
- 589 70. **Fernandez Guerrero ML, Ramos JM, Nunez A, Cuenca M, de Gorgolas M.**  
590 1997. Focal infections due to non-typhi Salmonella in patients with AIDS:  
591 report of 10 cases and review. *Clin Infect Dis* **25**:690-697.
- 592 71. **van Sorge NM, Doran KS.** 2012. Defense at the border: the blood-brain  
593 barrier versus bacterial foreigners. *Future Microbiol* **7**:383-394.
- 594 72. **Melican K, Michea Veloso P, Martin T, Bruneval P, Dumenil G.** 2013.  
595 Adhesion of Neisseria meningitidis to dermal vessels leads to local vascular

- 596 damage and purpura in a humanized mouse model. *PLoS Pathog*  
597 **9**:e1003139.
- 598 73. **Bonazzi D, Lo Schiavo V, Machata S, Djafer-Cherif I, Nivoit P, Manriquez**  
599 **V, Tanimoto H, Husson J, Henry N, Chate H, Voituriez R, Dumenil G.**  
600 2018. Intermittent Pili-Mediated Forces Fluidize Neisseria meningitidis  
601 Aggregates Promoting Vascular Colonization. *Cell*  
602 doi:10.1016/j.cell.2018.04.010.
- 603 74. **Mairey E, Genovesio A, Donnadiou E, Bernard C, Jaubert F, Pinard E,**  
604 **Seylaz J, Olivo-Marin JC, Nassif X, Dumenil G.** 2006. Cerebral  
605 microcirculation shear stress levels determine Neisseria meningitidis  
606 attachment sites along the blood-brain barrier. *J Exp Med* **203**:1939-1950.
- 607 75. **Faust SN, Levin M, Harrison OB, Goldin RD, Lockhart MS, Kondaveeti S,**  
608 **Laszik Z, Esmon CT, Heyderman RS.** 2001. Dysfunction of endothelial  
609 protein C activation in severe meningococcal sepsis. *N Engl J Med* **345**:408-  
610 416.
- 611 76. **Imhaus AF, Dumenil G.** 2014. The number of Neisseria meningitidis type IV  
612 pili determines host cell interaction. *EMBO J* **33**:1767-1783.
- 613 77. **Bernard SC, Simpson N, Join-Lambert O, Federici C, Laran-Chich MP,**  
614 **Maissa N, Bouzinba-Segard H, Morand PC, Chretien F, Taouji S, Chevet**  
615 **E, Janel S, Lafont F, Coureuil M, Segura A, Niedergang F, Marullo S,**  
616 **Couraud PO, Nassif X, Bourdoulous S.** 2014. Pathogenic Neisseria  
617 meningitidis utilizes CD147 for vascular colonization. *Nat Med* **20**:725-731.
- 618 78. **Maissa N, Covarelli V, Janel S, Durel B, Simpson N, Bernard SC, Pardo-**  
619 **Lopez L, Bouzinba-Segard H, Faure C, Scott MGH, Coureuil M, Morand**  
620 **PC, Lafont F, Nassif X, Marullo S, Bourdoulous S.** 2017. Strength of  
621 Neisseria meningitidis binding to endothelial cells requires highly-ordered  
622 CD147/beta2-adrenoceptor clusters assembled by alpha-actinin-4. *Nat*  
623 *Commun* **8**:15764.
- 624 79. **Eugene E, Hoffmann I, Pujol C, Couraud PO, Bourdoulous S, Nassif X.**  
625 2002. Microvilli-like structures are associated with the internalization of virulent  
626 capsulated Neisseria meningitidis into vascular endothelial cells. *J Cell Sci*  
627 **115**:1231-1241.
- 628 80. **Soyer M, Charles-Orszag A, Lagache T, Machata S, Imhaus AF, Dumont**  
629 **A, Millien C, Olivo-Marin JC, Dumenil G.** 2014. Early sequence of events  
630 triggered by the interaction of Neisseria meningitidis with endothelial cells. *Cell*  
631 *Microbiol* **16**:878-895.
- 632 81. **Mikaty G, Soyer M, Mairey E, Henry N, Dyer D, Forest KT, Morand P,**  
633 **Guadagnini S, Prevost MC, Nassif X, Dumenil G.** 2009. Extracellular  
634 bacterial pathogen induces host cell surface reorganization to resist shear  
635 stress. *PLoS Pathog* **5**:e1000314.
- 636 82. **Merz AJ, So M.** 1997. Attachment of piliated, Opa- and Opc- gonococci and  
637 meningococci to epithelial cells elicits cortical actin rearrangements and  
638 clustering of tyrosine-phosphorylated proteins. *Infect Immun* **65**:4341-4349.
- 639 83. **Coureuil M, Mikaty G, Miller F, Lecuyer H, Bernard C, Bourdoulous S,**  
640 **Dumenil G, Mege RM, Weksler BB, Romero IA, Couraud PO, Nassif X.**  
641 2009. Meningococcal type IV pili recruit the polarity complex to cross the brain  
642 endothelium. *Science* **325**:83-87.
- 643 84. **Hoehn B, Duval X.** 2013. Infective endocarditis. *N Engl J Med* **369**:785.
- 644 85. **Cahill TJ, Prendergast BD.** 2016. Infective endocarditis. *Lancet* **387**:882-  
645 893.

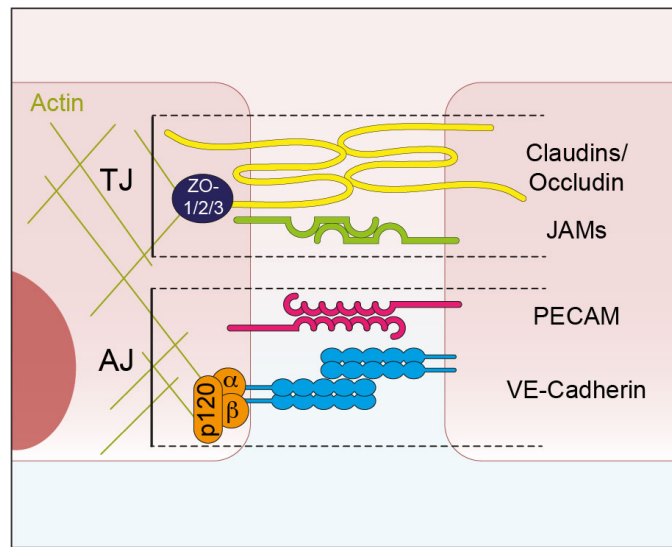
- 646 86. **Vilcant V, Hai O.** 2018. Endocarditis, Bacterial, *StatPearls*, Treasure Island  
647 (FL).
- 648 87. **McDonald JR, Olaison L, Anderson DJ, Hoen B, Miro JM, Eykyn S,**  
649 **Abrutyn E, Fowler VG, Jr., Habib G, Selton-Suty C, Pappas PA, Cabell**  
650 **CH, Corey GR, Marco F, Sexton DJ.** 2005. Enterococcal endocarditis: 107  
651 cases from the international collaboration on endocarditis merged database.  
652 *Am J Med* **118**:759-766.
- 653 88. **Yew HS, Murdoch DR.** 2012. Global trends in infective endocarditis  
654 epidemiology. *Curr Infect Dis Rep* **14**:367-372.
- 655 89. **Widmer E, Que YA, Entenza JM, Moreillon P.** 2006. New concepts in the  
656 pathophysiology of infective endocarditis. *Curr Infect Dis Rep* **8**:271-279.
- 657 90. **Hemler ME, Elices MJ, Parker C, Takada Y.** 1990. Structure of the integrin  
658 VLA-4 and its cell-cell and cell-matrix adhesion functions. *Immunol Rev*  
659 **114**:45-65.
- 660 91. **Foster TJ, Hook M.** 1998. Surface protein adhesins of *Staphylococcus*  
661 *aureus*. *Trends Microbiol* **6**:484-488.
- 662 92. **Chavakis T, Wiechmann K, Preissner KT, Herrmann M.** 2005.  
663 *Staphylococcus aureus* interactions with the endothelium: the role of bacterial  
664 "secretable expanded repertoire adhesive molecules" (SERAM) in disturbing  
665 host defense systems. *Thromb Haemost* **94**:278-285.
- 666 93. **Moreillon P, Que YA, Bayer AS.** 2002. Pathogenesis of streptococcal and  
667 staphylococcal endocarditis. *Infect Dis Clin North Am* **16**:297-318.
- 668 94. **Werdan K, Dietz S, Loffler B, Niemann S, Bushnaq H, Silber RE, Peters G,**  
669 **Muller-Werdan U.** 2014. Mechanisms of infective endocarditis: pathogen-host  
670 interaction and risk states. *Nat Rev Cardiol* **11**:35-50.
- 671 95. **Haslinger-Loffler B, Kahl BC, Grundmeier M, Strangfeld K, Wagner B,**  
672 **Fischer U, Cheung AL, Peters G, Schulze-Osthoff K, Sinha B.** 2005.  
673 Multiple virulence factors are required for *Staphylococcus aureus*-induced  
674 apoptosis in endothelial cells. *Cell Microbiol* **7**:1087-1097.
- 675 96. **Brouqui P, Raoult D.** 2001. Endocarditis due to rare and fastidious bacteria.  
676 *Clin Microbiol Rev* **14**:177-207.
- 677 97. **Fournier PE, Lelievre H, Eykyn SJ, Mainardi JL, Marrie TJ, Bruneel F,**  
678 **Roure C, Nash J, Clave D, James E, Benoit-Lemercier C, Deforges L,**  
679 **Tissot-Dupont H, Raoult D.** 2001. Epidemiologic and clinical characteristics  
680 of *Bartonella quintana* and *Bartonella henselae* endocarditis: a study of 48  
681 patients. *Medicine (Baltimore)* **80**:245-251.
- 682 98. **Raoult D, Fournier PE, Vandenesch F, Mainardi JL, Eykyn SJ, Nash J,**  
683 **James E, Benoit-Lemercier C, Marrie TJ.** 2003. Outcome and treatment of  
684 *Bartonella* endocarditis. *Arch Intern Med* **163**:226-230.
- 685 99. **Raoult D, Fournier PE, Drancourt M, Marrie TJ, Etienne J, Cosserat J,**  
686 **Cacoub P, Poinsignon Y, Leclercq P, Sefton AM.** 1996. Diagnosis of 22  
687 new cases of *Bartonella* endocarditis. *Ann Intern Med* **125**:646-652.
- 688 100. **Dehio C.** 2004. Molecular and cellular basis of *Bartonella* pathogenesis. *Annu*  
689 *Rev Microbiol* **58**:365-390.
- 690 101. **Breitschwerdt EB, Kordick DL.** 2000. *Bartonella* infection in animals:  
691 carriership, reservoir potential, pathogenicity, and zoonotic potential for human  
692 infection. *Clin Microbiol Rev* **13**:428-438.
- 693 102. **Chomel BB.** 2000. Cat-scratch disease. *Rev Sci Tech* **19**:136-150.



- 694 103. **Koehler JE, Tappero JW.** 1993. Bacillary angiomatosis and bacillary peliosis  
695 in patients infected with human immunodeficiency virus. *Clin Infect Dis*  
696 **17**:612-624.
- 697 104. **Regnery RL, Childs JE, Koehler JE.** 1995. Infections associated with  
698 *Bartonella* species in persons infected with human immunodeficiency virus.  
699 *Clin Infect Dis* **21 Suppl 1**:S94-98.
- 700 105. **Kostianovsky M, Greco MA.** 1994. Angiogenic process in bacillary  
701 angiomatosis. *Ultrastruct Pathol* **18**:349-355.
- 702 106. **Manders SM.** 1996. Bacillary angiomatosis. *Clin Dermatol* **14**:295-299.
- 703 107. **Dehio C, Meyer M, Berger J, Schwarz H, Lanz C.** 1997. Interaction of  
704 *Bartonella henselae* with endothelial cells results in bacterial aggregation on  
705 the cell surface and the subsequent engulfment and internalisation of the  
706 bacterial aggregate by a unique structure, the invasome. *J Cell Sci* **110 ( Pt**  
707 **18)**:2141-2154.
- 708 108. **Verma A, Davis GE, Ihler GM.** 2000. Infection of human endothelial cells with  
709 *Bartonella bacilliformis* is dependent on Rho and results in activation of Rho.  
710 *Infect Immun* **68**:5960-5969.
- 711 109. **Dramsi S, Cossart P.** 1998. Intracellular pathogens and the actin  
712 cytoskeleton. *Annu Rev Cell Dev Biol* **14**:137-166.
- 713 110. **Kirby JE, Nekorchuk DM.** 2002. *Bartonella*-associated endothelial  
714 proliferation depends on inhibition of apoptosis. *Proc Natl Acad Sci U S A*  
715 **99**:4656-4661.
- 716 111. **Schulein R, Dehio C.** 2002. The VirB/VirD4 type IV secretion system of  
717 *Bartonella* is essential for establishing intraerythrocytic infection. *Mol Microbiol*  
718 **46**:1053-1067.
- 719 112. **Seubert A, Hiestand R, de la Cruz F, Dehio C.** 2003. A bacterial conjugation  
720 machinery recruited for pathogenesis. *Mol Microbiol* **49**:1253-1266.
- 721 113. **Torisu H, Ono M, Kiryu H, Furue M, Ohmoto Y, Nakayama J, Nishioka Y,**  
722 **Sone S, Kuwano M.** 2000. Macrophage infiltration correlates with tumor stage  
723 and angiogenesis in human malignant melanoma: possible involvement of  
724 TNFalpha and IL-1alpha. *Int J Cancer* **85**:182-188.
- 725 114. **Musso T, Badolato R, Ravarino D, Stornello S, Panzanelli P, Merlino C,**  
726 **Savoia D, Cavallo R, Ponzi AN, Zucca M.** 2001. Interaction of *Bartonella*  
727 *henselae* with the murine macrophage cell line J774: infection and  
728 proinflammatory response. *Infect Immun* **69**:5974-5980.
- 729 115. **Kempf VA, Volkmann B, Schaller M, Sander CA, Alitalo K, Riess T,**  
730 **Autenrieth IB.** 2001. Evidence of a leading role for VEGF in *Bartonella*  
731 *henselae*-induced endothelial cell proliferations. *Cell Microbiol* **3**:623-632.
- 732 116. **Resto-Ruiz SI, Schmiederer M, Sweger D, Newton C, Klein TW, Friedman**  
733 **H, Anderson BE.** 2002. Induction of a potential paracrine angiogenic loop  
734 between human THP-1 macrophages and human microvascular endothelial  
735 cells during *Bartonella henselae* infection. *Infect Immun* **70**:4564-4570.
- 736 117. **Minasyan H.** 2016. Mechanisms and pathways for the clearance of bacteria  
737 from blood circulation in health and disease. *Pathophysiology* **23**:61-66.
- 738 118. **Khakpour S, Wilhelmsen K, Hellman J.** 2015. Vascular endothelial cell Toll-  
739 like receptor pathways in sepsis. *Innate Immun* **21**:827-846.
- 740 119. **Gupta SK, Lysko PG, Pillarisetti K, Ohlstein E, Stadel JM.** 1998.  
741 Chemokine receptors in human endothelial cells. Functional expression of  
742 CXCR4 and its transcriptional regulation by inflammatory cytokines. *J Biol*  
743 *Chem* **273**:4282-4287.

- 744 120. **Murdoch C, Monk PN, Finn A.** 1999. Cxc chemokine receptor expression on  
745 human endothelial cells. *Cytokine* **11**:704-712.
- 746 121. **Mitchell JA, Ryffel B, Quesniaux VF, Cartwright N, Paul-Clark M.** 2007.  
747 Role of pattern-recognition receptors in cardiovascular health and disease.  
748 *Biochem Soc Trans* **35**:1449-1452.
- 749 122. **Opitz B, Eitel J, Meixenberger K, Suttorp N.** 2009. Role of Toll-like  
750 receptors, NOD-like receptors and RIG-I-like receptors in endothelial cells and  
751 systemic infections. *Thromb Haemost* **102**:1103-1109.
- 752 123. **Marceau F, Grassi J, Frobert Y, Bergeron C, Poubelle PE.** 1992. Effects of  
753 experimental conditions on the production of interleukin-1 alpha and -1 beta by  
754 human endothelial cells cultured in vitro. *Int J Immunopharmacol* **14**:525-534.
- 755 124. **Opitz B, Puschel A, Beermann W, Hocke AC, Forster S, Schmeck B, van  
756 Laak V, Chakraborty T, Suttorp N, Hippenstiel S.** 2006. Listeria  
757 monocytogenes activated p38 MAPK and induced IL-8 secretion in a  
758 nucleotide-binding oligomerization domain 1-dependent manner in endothelial  
759 cells. *J Immunol* **176**:484-490.
- 760 125. **Anand AR, Cucchiaroni M, Terwilliger EF, Ganju RK.** 2008. The tyrosine  
761 kinase Pyk2 mediates lipopolysaccharide-induced IL-8 expression in human  
762 endothelial cells. *J Immunol* **180**:5636-5644.
- 763 126. **Faure E, Thomas L, Xu H, Medvedev A, Equils O, Arditi M.** 2001. Bacterial  
764 lipopolysaccharide and IFN-gamma induce Toll-like receptor 2 and Toll-like  
765 receptor 4 expression in human endothelial cells: role of NF-kappa B  
766 activation. *J Immunol* **166**:2018-2024.
- 767 127. **Danese S, Dejana E, Fiocchi C.** 2007. Immune regulation by microvascular  
768 endothelial cells: directing innate and adaptive immunity, coagulation, and  
769 inflammation. *J Immunol* **178**:6017-6022.
- 770 128. **Neefjes J, Jongstra ML, Paul P, Bakke O.** 2011. Towards a systems  
771 understanding of MHC class I and MHC class II antigen presentation. *Nat Rev*  
772 *Immunol* **11**:823-836.
- 773 129. **Rose ML, Coles MI, Griffin RJ, Pomerance A, Yacoub MH.** 1986.  
774 Expression of class I and class II major histocompatibility antigens in normal  
775 and transplanted human heart. *Transplantation* **41**:776-780.
- 776 130. **Leeuwenberg JF, Van Damme J, Meager T, Jeunhomme TM, Buurman  
777 WA.** 1988. Effects of tumor necrosis factor on the interferon-gamma-induced  
778 major histocompatibility complex class II antigen expression by human  
779 endothelial cells. *Eur J Immunol* **18**:1469-1472.
- 780 131. **Mai J, Virtue A, Shen J, Wang H, Yang XF.** 2013. An evolving new  
781 paradigm: endothelial cells--conditional innate immune cells. *J Hematol Oncol*  
782 **6**:61.
- 783 132. **van der Poll T, van de Veerdonk FL, Scicluna BP, Netea MG.** 2017. The  
784 immunopathology of sepsis and potential therapeutic targets. *Nat Rev*  
785 *Immunol* **17**:407-420.
- 786 133. **Wiersinga WJ, Leopold SJ, Cranendonk DR, van der Poll T.** 2014. Host  
787 innate immune responses to sepsis. *Virulence* **5**:36-44.
- 788 134. **Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J,  
789 Opal SM, Vincent JL, Ramsay G, Sccm/Esicm/Accp/Ats/Sis.** 2003. 2001  
790 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference.  
791 *Crit Care Med* **31**:1250-1256.
- 792 135. **Rittirsch D, Flierl MA, Ward PA.** 2008. Harmful molecular mechanisms in  
793 sepsis. *Nat Rev Immunol* **8**:776-787.

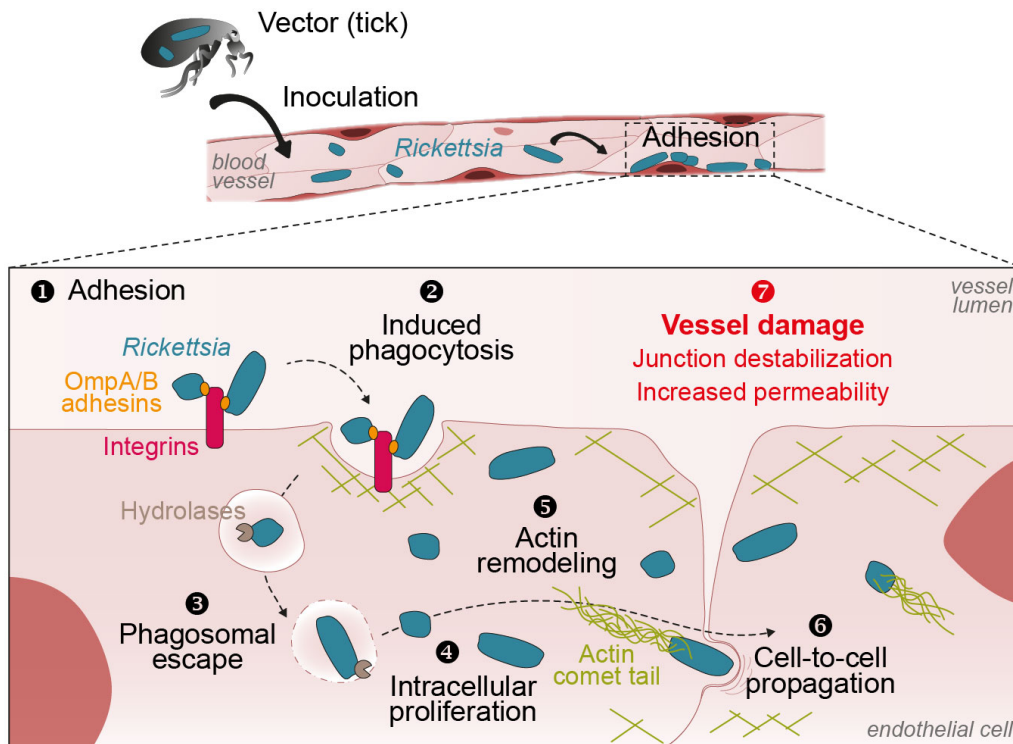
- 794 136. **Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent**  
795 **JL.** 2016. Sepsis and septic shock. *Nat Rev Dis Primers* **2**:16045.
- 796 137. **Hotchkiss RS, Tinsley KW, Swanson PE, Grayson MH, Osborne DF,**  
797 **Wagner TH, Cobb JP, Coopersmith C, Karl IE.** 2002. Depletion of dendritic  
798 cells, but not macrophages, in patients with sepsis. *J Immunol* **168**:2493-2500.
- 799 138. **Hotchkiss RS, Monneret G, Payen D.** 2013. Sepsis-induced  
800 immunosuppression: from cellular dysfunctions to immunotherapy. *Nat Rev*  
801 *Immunol* **13**:862-874.
- 802 139. **Scumpia PO, Delano MJ, Kelly-Scumpia KM, Weinstein JS, Wynn JL,**  
803 **Winfield RD, Xia C, Chung CS, Ayala A, Atkinson MA, Reeves WH, Clare-**  
804 **Salzler MJ, Moldawer LL.** 2007. Treatment with GITR agonistic antibody  
805 corrects adaptive immune dysfunction in sepsis. *Blood* **110**:3673-3681.
- 806 140. **Boomer JS, To K, Chang KC, Takasu O, Osborne DF, Walton AH, Bricker**  
807 **TL, Jarman SD, 2nd, Kreisel D, Krupnick AS, Srivastava A, Swanson PE,**  
808 **Green JM, Hotchkiss RS.** 2011. Immunosuppression in patients who die of  
809 sepsis and multiple organ failure. *JAMA* **306**:2594-2605.
- 810 141. **Huang X, Venet F, Wang YL, Lepape A, Yuan Z, Chen Y, Swan R, Kherouf**  
811 **H, Monneret G, Chung CS, Ayala A.** 2009. PD-1 expression by  
812 macrophages plays a pathologic role in altering microbial clearance and the  
813 innate inflammatory response to sepsis. *Proc Natl Acad Sci U S A* **106**:6303-  
814 6308.
- 815 142. **Levi M, Ten Cate H.** 1999. Disseminated intravascular coagulation. *N Engl J*  
816 *Med* **341**:586-592.
- 817 143. **Davis RP, Miller-Dorey S, Jenne CN.** 2016. Platelets and coagulation in  
818 infection. *Clin Transl Immunology* **5**:e89.
- 819 144. **Pernerstorfer T, Stohlawetz P, Hollenstein U, Dzirlo L, Eichler HG,**  
820 **Kapiotis S, Jilma B, Speiser W.** 1999. Endotoxin-induced activation of the  
821 coagulation cascade in humans: effect of acetylsalicylic acid and  
822 acetaminophen. *Arterioscler Thromb Vasc Biol* **19**:2517-2523.
- 823 145. **Osterud B, Bjorklid E.** 2001. The tissue factor pathway in disseminated  
824 intravascular coagulation. *Semin Thromb Hemost* **27**:605-617.
- 825 146. **Abraham E.** 2000. Coagulation abnormalities in acute lung injury and sepsis.  
826 *Am J Respir Cell Mol Biol* **22**:401-404.
- 827 147. **Ince C.** 2005. The microcirculation is the motor of sepsis. *Crit Care* **9 Suppl**  
828 **4**:S13-19.
- 829



830

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

840



841

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,

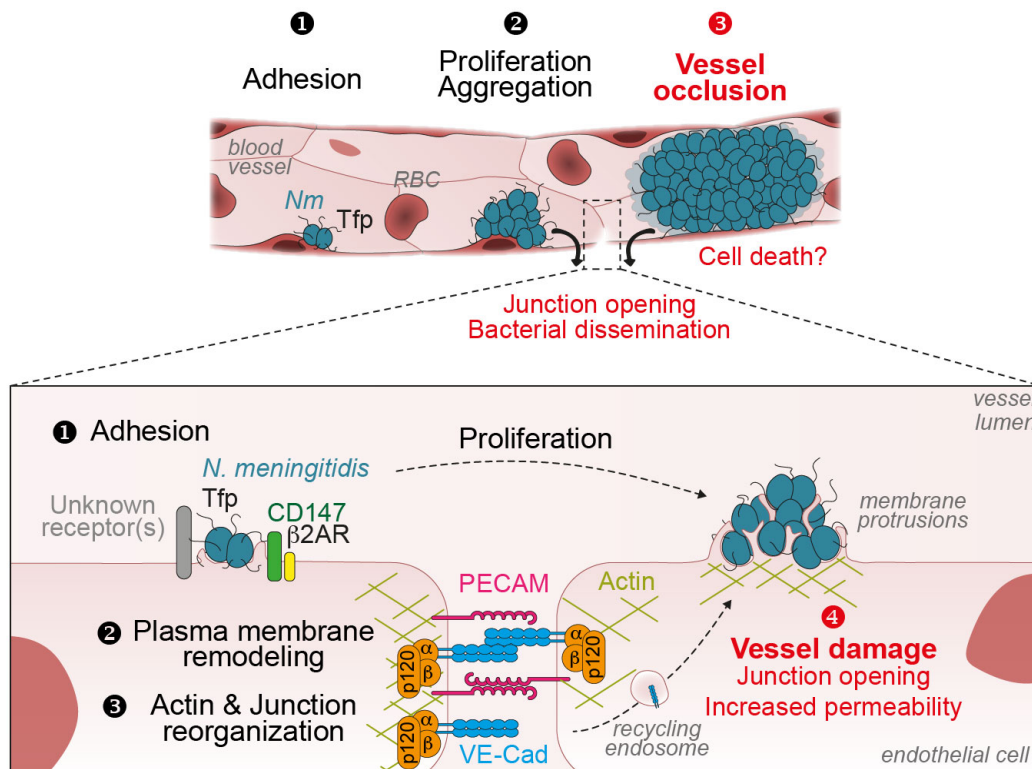
849 to assemble actin comet tails supporting bacterial movement and cell-to-cell

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.

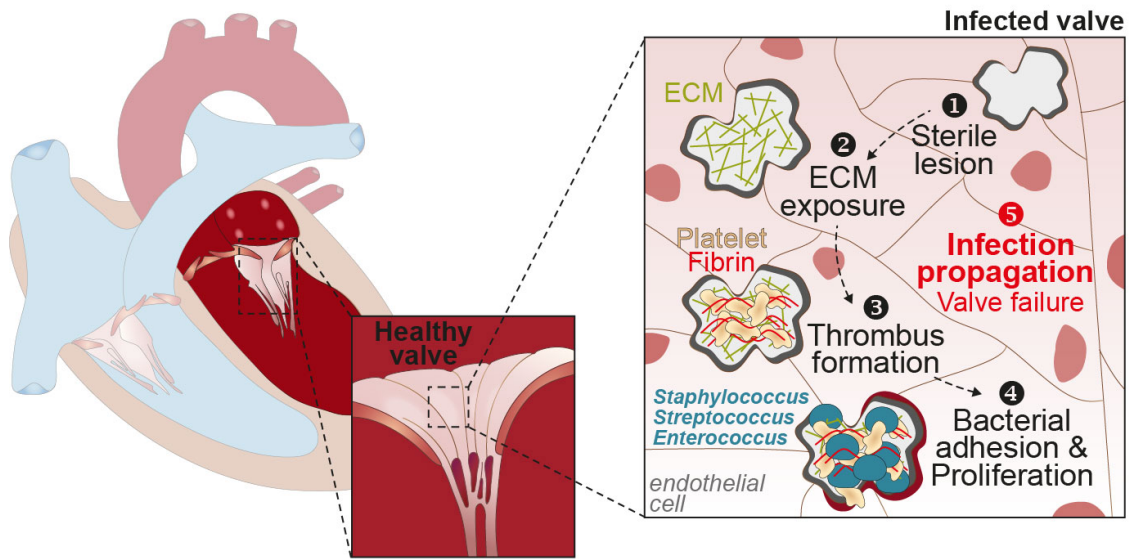
853



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  $\beta$ 2-adrenergic receptor ( $\beta$ 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

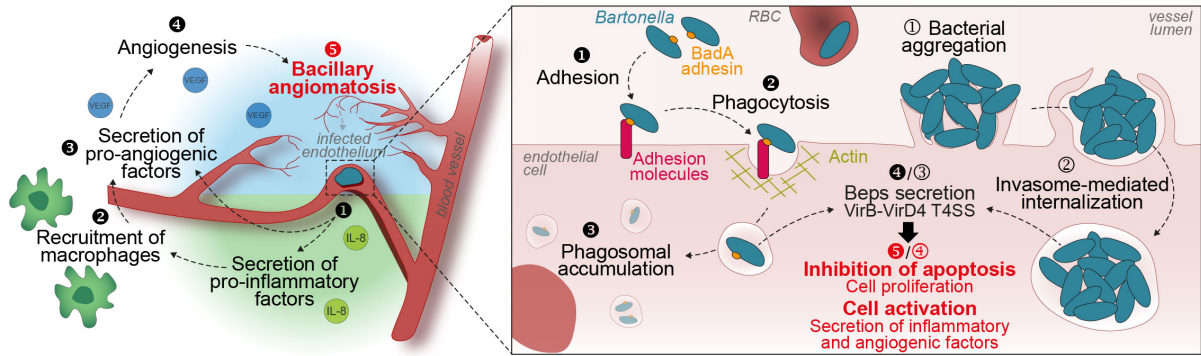


869

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





879

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