

Breaking the wall: targeting of the endothelium by pathogenic bacteria

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Abstract | The endothelium lining blood and lymphatic vessels is a key barrier separating body fluids from host tissues and is a major target of pathogenic bacteria. Endothelial cells are actively involved in host responses to infectious agents, producing inflammatory cytokines, controlling coagulation cascades and regulating leukocyte trafficking. In this Review, a range of bacteria and bacterial toxins are used to illustrate how pathogens establish intimate interactions with endothelial cells, triggering inflammatory responses and coagulation processes and modifying endothelial cell plasma membranes and junctions to adhere to their surfaces and then invade, cross and even disrupt the endothelial barrier.

The steady increase in the human lifespan over the past few decades largely reflects the major advances that have been made in controlling bacterial infections through the improvement of hygiene and the implementation of vaccination and antimicrobial therapy. However, the fight against microbial infections that target the cardiovascular system remains a major challenge for public health (for viral infections, see REF. 1). Endothelium forms the inner cellular lining of blood and lymphatic vessels, covering a large surface area of 4,000 to 7,000 m² and consisting of 10¹³ endothelial cells in a typical adult human². Far from being a homogeneous and inert surface, the endothelium shows sharp structural and functional heterogeneities^{3,4} (FIG. 1). As a result of organ-specific requirements, the endothelial cell-to-cell junctions, which are vital for the development and integrity of blood vessels, exhibit considerable variability in their organization along the vascular system: tight junctions are well developed in brain microvessels — where they contribute to the blood–brain barrier — and also in arteries, whereas they are less organized in veins, capillaries and organs that are characterized by a high rate of exchange⁵. This strict regulation of endothelial barrier function reflects the physiological exchange of a diverse range of metabolites, hormones and gases, as well as leukocytes, between organs and controls blood pressure and flow to ensure body homeostasis^{3,4}. In addition, endothelial cells perceive pathogens and inflammatory signals and are key regulators of inflammation and haemostasis⁶.

Human bacterial pathogens that colonize and sometimes breach the skin or mucosal epithelia or that are injected beneath the skin epithelial barrier by insect

vectors are a cause of bloodborne infections. Such pathogens can adhere to the endothelial walls of vessels, which are widely distributed in all tissues and are therefore the preferred interface for systemic dissemination of bacteria. Moreover, dysfunction, rupture and breaching of the endothelial barrier provide highly effective means of bacterial dissemination into tissues, leading to major pathologies (BOX 1).

A striking illustration of the health threat presented by bloodborne infections is the frequency of severe sepsis, which results from bacteria-induced endothelial dysfunction (see below) and is estimated to cause up to 9.3% of all deaths in the United States⁷ (BOX 1). Recent advances in our understanding of the pathophysiology of sepsis and toxic shock have highlighted both host and bacterial genetic contributions to the variability of inflammatory responses between individuals. Furthermore, in addition to sepsis, infections can cause other severe conditions, including endocarditis and focal vascular infection, septicaemia and ensuing septic metastasis and visceral abscesses, and focal complications of bloodborne infections (for example, arthritis, meningitis and fetoplacental infections), as well as the formation of atherogenic or neo-angiogenic lesions and chronic infections that are thought to favour chronic systemic inflammation (TABLE 1). However, the suggestion that pathogenic bacteria such as *Chlamydia pneumoniae* are implicated in cardiovascular diseases of inflammatory origin has not yet been formally demonstrated⁸.

A wide array of molecules are produced by pathogenic bacteria to enable them to adhere to and damage the endothelium, resist the shear stress exerted by the

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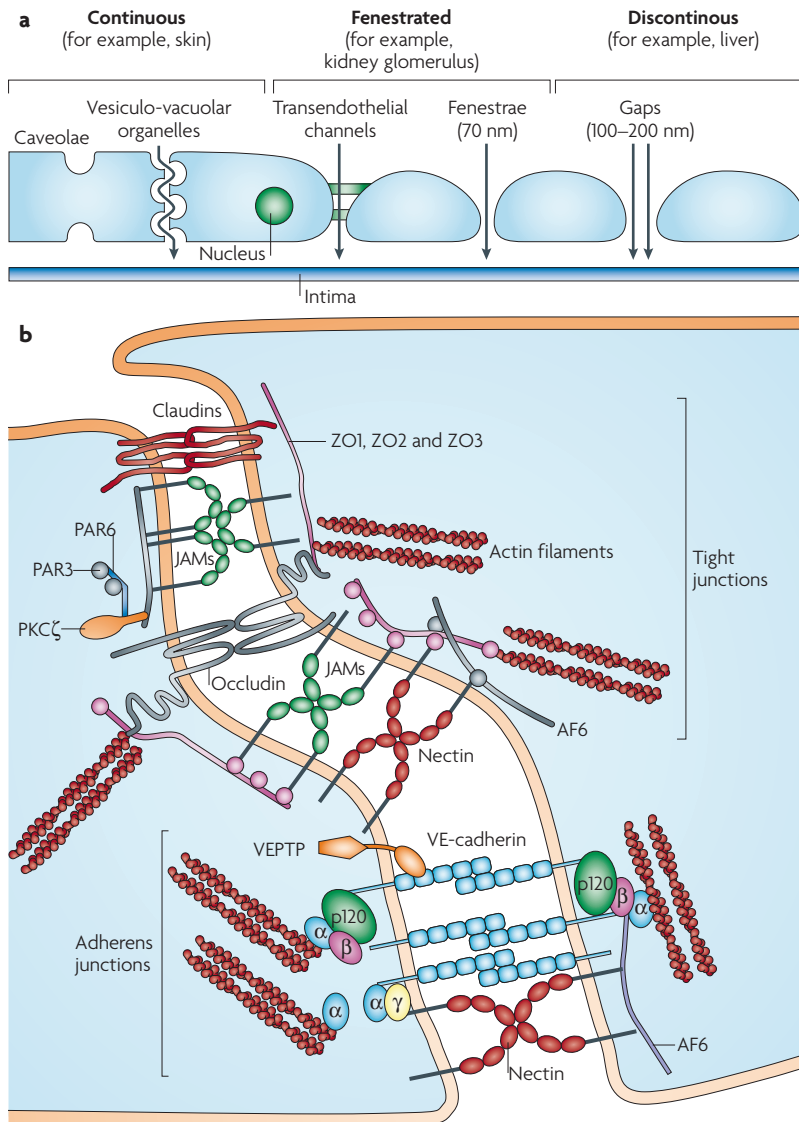


Figure 1 | Structural features of the endothelial barrier. a | To foster exchanges, endothelial cells can be extremely thin ($\leq 0.1 \mu\text{m}$) and enriched in caveolae (70 nm, flask-shaped vesicles). Capillary endothelial cells bear various channels and discontinuities, including vesiculo-vacuolar organelles (formed by the coalescence of caveolin 1-containing vesicles and vacuoles), transendothelial channels, fenestrae (70 nm diameter channels), with or without non-membranous diaphragms containing plasmalemma vesicle protein 1, and large gaps (100–200 nm in diameter). **b** | Adherens junctions are promoted by vascular endothelial (VE) cadherin. Cadherins bind directly to p120 catenin (also known as $\delta 1$ catenin) (p120), β -catenin (β), junction plakoglobin (also known as γ -catenin) (γ) and α -catenin (α), which binds to actin microfilaments. VE protein tyrosine phosphatase (VEPTP) controls adherens junction integrity. Tight junctions are promoted by claudins, with the cooperation of occludin and junctional adhesion molecules (JAMs). Zonula occludens 1 (ZO1; also known as TJP1), ZO2 (also known as TJP2) and cingulin contribute to tight junction interaction with the actin cytoskeleton. ZO1 also associates with claudins, JAMs, occludin, ZO2 and ZO3 (also known as TJP3) and directly binds to F-actin, allowing the formation of large protein complexes. Brain endothelial tight junctions express several different claudins (CLDN1, CLDN3, CLDN5 and CLDN12), but only CLDN5 is endothelial cell-specific and actively contributes to blood–brain barrier integrity. JAM family members associate with tight junction strands through interactions with PDZ domain-containing proteins, including ZO1, ZO2, AF6 (also known as afadin) and partitioning-defective protein 3 (PAR3). The PAR3–PAR6–protein kinase C ζ (PKC ζ) polarity complex plays a central part in the formation of tight junctions and cell polarity. Nectins and their intracellular partner AF6 participate in the organization of both tight and adherens junctions.

blood flow at the blood–endothelial interface, avoid or promote their uptake by endothelial cells, disseminate in tissues and manipulate systemic host innate and adaptive immune responses (FIGS 2,3). The identification of some of these key factors has triggered intensive research and led to notable advances in our understanding of the interplay between microorganisms and the endothelium and of its pathogenic consequences.

In this Review, we highlight novel aspects of the endothelial dysfunctions that are induced by bacterial pathogens and discuss bacterial strategies to promote adhesion, invasion and breaching of endothelial barriers. We also present recent discoveries about the impact of bacterial toxins on endothelial cell survival and actin cytoskeleton rearrangements.

Endothelial responses and dysfunctions

Systemic inflammatory responses to infection, or sepsis (BOX 1), are an immediate consequence of bacterial access to and multiplication in the bloodstream following a breach of the skin or mucosal epithelia by a wound, an insect bite or active translocation (TABLE 1). Endothelial cells actively participate in inflammatory responses and control coagulation cascades^{6,9}. Severe sepsis is accompanied by organ dysfunctions and, sometimes, septic shock and is the most common cause of death among hospitalized patients in non-coronary intensive-care units⁷. Endothelial cells also respond to signals of danger, inflammatory mediators and conserved pathogen-associated molecular patterns (PAMPs) by controlling the permeability of the endothelium and upregulating adhesion factors for binding and extravasation of leukocytes through the intercellular and the transcellular routes (both of which have been recently characterized at the molecular level)^{10,11}.

Endothelium inflammatory responses to pathogens.

The progression of sepsis severity is now seen as an array of inappropriate host responses following the perception of danger signals or triggered by toxins, and it is considered to be a consequence of host and bacterial genetic determinants¹². Studies of vascular and lymphatic endothelial cells at different sites in the vascular system show that they express large repertoires of functional PAMP receptors, such as the toll-like receptors (TLRs)^{13,14}. TLR1, TLR2, TLR4, TLR5 and TLR6 are involved in recognizing a series of bacterial PAMPs at the cell surface, whereas TLR3, TLR7, TLR8 and TLR9 detect nucleic acids in intracellular compartments. The intensity of the cellular responses of vascular and lymphatic endothelia to PAMPs may vary depending on their anatomical origin and does not strictly correlate with TLR expression levels, with higher overall responses obtained for dermal endothelial cells¹³. This possibly reflects the lower sensitivity of the skin to the detrimental effects of high inflammatory responses and the need to efficiently circumvent frequent infections at this barrier interface. More generally, the responsiveness of endothelial cell populations to specific PAMPs is indicative of their capacity to recognize bacteria and

Box 1 | Cardiovascular-associated pathologies*

Infection

A microbial phenomenon that is characterized by an inflammatory reaction to the presence of microorganisms or the invasion of normally sterile host tissue by those organisms.

Bacteraemia

The presence of viable bacteria in the blood.

SIRS

The systemic inflammatory response syndrome (SIRS) is the response to a range of severe clinical insults; the SIRS response to infection is called sepsis. The response manifests as two or more of the following conditions: a body temperature of $>38^{\circ}\text{C}$ or $<36^{\circ}\text{C}$; a heart rate of >90 beats per minute; a respiratory rate of >20 breaths per minute or a PaCO_2 (partial pressure of CO_2 in the blood) of <32 mm Hg; and a white blood cell count of $>12,000\text{ mm}^3$, $<4,000\text{ mm}^3$ or $>10\%$ immature form (or band form) cells.

Severe sepsis

Sepsis associated with organ dysfunction, hypoperfusion or hypotension. Hypoperfusion and perfusion abnormalities can include, but are not limited to, lactic acidose, oliguria or an acute alteration in mental status.

Septic shock

Sepsis with hypotension, despite adequate fluid resuscitation, along with the occurrence of perfusion abnormalities. Patients who are receiving inotropic or vasopressor agents may not be hypotensive at the time that perfusion abnormalities are measured.

Sepsis-induced hypotension

A systolic blood pressure of <90 mm Hg or a reduction of ≥ 40 mm Hg from baseline in the absence of other causes for hypotension.

Multiple organ dysfunction syndrome

Altered organ function in an acutely ill patient, such that homeostasis cannot be maintained without intervention.

HUS and TTP

Haemolytic uraemia syndrome (HUS) and thrombocytopenic purpura (TTP) are acute syndromes with multiple organ dysfunctions and evidencing microangiopathic haemolytic anaemia and thrombocytopenia. The disorder is considered to be HUS when acute renal failure is dominant and, inversely, TTP when the neurological disorders are dominant.

Atherosclerosis

During atherogenesis, endothelial cells activated by inflammatory signals trigger the recruitment and adhesion of monocytes and macrophages, as well as their transmigration into the subendothelium intima. Mature atheromatous plaques, the formation of which is promoted by cholesterol accumulation, can impede blood flow or trigger the formation of a thrombus on plaque rupture or erosion, leading to infarctions and strokes.

*Information from REF. 114

viruses. Recognition of PAMPs by the extracellular domains of the TLRs on both endothelial and immune cells triggers the expression of pro-inflammatory factors through the initiation of signal transduction pathways that lead to the activation of mitogen-activated protein (MAP) kinases such as p38 and JUN amino-terminal kinase (Jnk) proteins, as well as nuclear factor- κB (NF- κB) (for reviews, see REFS. 15,16). Rho GTPases have emerged as important regulators of the expression of inflammatory genes, owing to their ability to activate Jnk proteins, p38 and NF- κB downstream of TLRs¹⁷. Rho GTPases are targets of numerous virulence factors of both Gram-negative and Gram-positive pathogenic bacteria¹⁸. Activation of Rho proteins by bacterial virulence factors triggers the production of inflammatory mediators in endothelial cells, as exemplified by the response to direct activation of Rho proteins by the cytotoxic necrotizing factor 1 (CNF1) toxin of uropathogenic *Escherichia coli* (UPEC), a pathogen that is frequently associated with bacteraemia^{19–21}. To date, major inducers of septic shock include the endotoxin, which refers to the lipid A component of the lipopolysaccharide (LPS) of Gram-negative bacteria, and superantigen

proteins that are produced by various Gram-positive cocci as well as by Gram-negative bacteria such as *Yersinia pseudotuberculosis*^{22,23}. Lipid A is an essential bacterial outer membrane component, the structure of which varies both between bacterial species and within species according to serotype and environmental conditions²². Endotoxins are recognized primarily by the complex formed by MD2 (also known as LY96), TLR4 and CD14 (REF. 22). Polymorphism in human TLR4 modulates the efficiency of recognition of various types of endotoxin and the extent of the inflammatory response²². Individuals with a less responsive TLR4 have a higher incidence of infections by Gram-negative bacteria and of septic shock. However, they also have a lower risk of atherogenesis, which is probably due to less severe inflammatory responses^{22,24}. Superantigen proteins of Gram-positive bacteria can trigger toxic shock syndrome associated with vascular dysfunctions. These toxins are mostly expressed by *Staphylococcus aureus* and *Streptococcus pyogenes*²³ and form a ternary complex with major histocompatibility complex class II molecules and T cell receptors (TCRs) to stimulate polyclonal activation of a subset of T cells that express a common TCR V β subunit, triggering

Atherogenesis

The process of atheromatous plaque formation during atherosclerosis.

Table 1 | Examples of pathogenic bacteria that target endothelial cells*

Bacteria	Disease	Main features	Contamination
<i>Bacillus anthracis</i>	Anthrax	Pneumonia, skin necrosis and toxic and haemorrhagic shock	Environment
<i>Bartonellae</i> spp.	Cat scratch disease and bacillary angiomatosis	Lymphadenopathy verruga peruana, endocarditis and angiomatosis–peliosis	Cat scratches
<i>Borrelia recurrentis</i>	Relapsing fever	Recurrent periods of fever and erythema	Lice and ticks
<i>Borrelia burgdorferi</i>	Lyme's disease	Fever, cardiac arrhythmia, facial paralysis, peripheral and central CNS symptoms and arthritis	Ticks
<i>Brucella</i> spp.	Brucellosis	Systemic infection with hepatosplenomegaly, high fever and sweating, and focal manifestations (endocarditis and joint, bone and CNS localization)	Unpasteurized milk and contaminated meat
<i>Chlamydia pneumoniae</i>	Possible link with atherosclerosis	Present in some atheromatous lesions	Environment
<i>Coxiella burnetii</i>	Q fever	Fever, pneumonia and hepatitis to chronic infection-associated endocarditis	Airborne
<i>Ehrlichia</i> spp.	Human granulocytic ehrlichiosis	Fever and headache	Ticks
<i>Francisella tularensis</i>	Tularemia	Systemic infection with cutaneous ulcerations, lymphadenitis and pneumonia	Direct contact with contaminated animals and (rarely) tick bite
<i>Haemophilus influenzae</i>	Sepsis	Meningitis and endotoxic shock (purpura fulminans)	Nasopharyngeal colonization
<i>Neisseria meningitidis</i>	Meningococemia and cerebrospinal meningitis	Septic shock (purpura fulminans) and meningitis	Nasopharyngeal colonization
<i>Rickettsia conorii</i> and <i>Rickettsia rickettsii</i>	Mediterranean spotted fever and Rocky Mountain spotted fever	Spotted rash and fever	Ticks
<i>Rickettsia prowazekii</i>	Epidemic typhus	Spotted rash on the trunk spreading to the appendages and a high fever	Lice
<i>Rickettsia typhi</i>	Murine typhus	Spotted rash on the trunk spreading to the appendages and a high fever	Flea
<i>Staphylococcus aureus</i>	Septicemia	Toxic shock, endocarditis and localized infections (including of the bone, joints, lung, liver and spleen)	Cutaneous colonization
<i>Streptococcus pyogenes</i> (group A streptococci)	Rheumatic fever	Immunopathological responses leading to tissue damage (to the heart, kidney and CNS)	Cutaneous colonization

CNS, central nervous system. *For general information on infectious diseases and the endothelium as a target of microorganisms, see the review REF. 1.

an inflammatory storm²⁵. Recent advances show that uncontrolled inflammatory responses during sepsis can be rapidly followed by an anti-inflammatory and immunosuppressive state, referred to as 'immunoparalysis', which involves a T helper 2-type response^{12,26}. Apart from those patients who suffer from toxic shock syndrome or severe sepsis induced by *Neisseria meningitidis* (also known as the meningococcus), which is usually referred as purpura fulminans and which leads to intravascular coagulation and multiple organ dysfunctions, most patients succumb during this second phase of sepsis, owing to their impaired ability to fight either the primary infection or secondary episodes of infection. Immunoparalysis, which persists for days, also involves epigenetic reprogramming²⁷.

Bacteria, neutrophils and endothelial cells. Interactions of bacteria and also of activated neutrophils and platelets with the vasculature can have harmful consequences in the cascades of events that lead to endothelium damage during sepsis²⁸. A recent study has shown that the TLR4-dependent activation of platelets by LPS *in vitro* triggers their association with neutrophils that are adhering to the endothelium²⁹. In addition, platelets also seem to be required *in vivo* for the binding of neutrophils to small capillaries, or sinusoids, of the liver. This leads to robust neutrophil activation, inducing a potent and fast release of neutrophil extracellular traps (NETs). These are networks of extracellular fibres composed of chromatin and associated destructive granular proteins and which act

Inflammatory storm

An unspecific and massive release of inflammatory mediators.

Toxic shock syndrome

Septic shock manifestations that are induced by bacterial toxins.

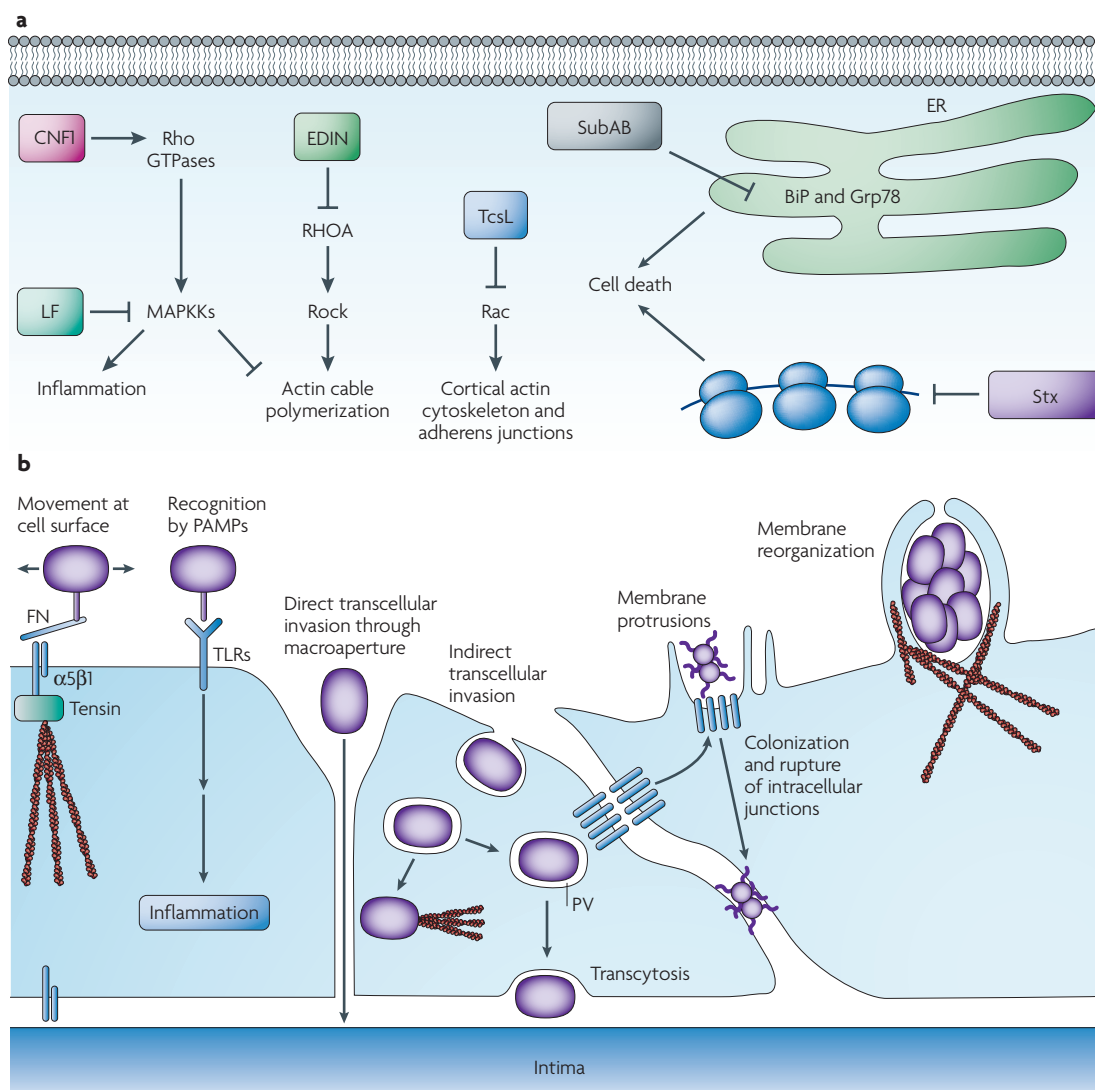


Figure 2 | Toxins and pathogens that target the endothelium. a | Bacterial toxins. Cytotoxic necrotizing factor 1 (CNF1) of uropathogenic *Escherichia coli* activates Rho GTPases, leading to the expression of inflammatory mediators¹⁹. Lethal factor (LF), a component of the lethal toxin of *Bacillus anthracis*, cleaves mitogen-activated protein kinase kinases (MAPKKs), blocking the expression of inflammatory mediators and triggering the formation of actin cables and the destabilization of intercellular junctions, as well as the induction of cell death^{99,100}. Epidermal-cell differentiation inhibitor (EDIN) of *Staphylococcus aureus* inactivates the GTPase RHOA, disrupting actin cables and inducing the formation of large tunnels⁶⁸ (see [Supplementary information S1](#) (movie)). The lethal toxin of *Clostridium Sordellii*, TcsL, triggers the inactivation of Rac and cell division cycle protein 42 (CDC42) (but not of RHOA), as well as inactivating other small GTPases of the Ras superfamily, leading to endothelial cell death⁹⁸. The Shiga-like toxins (Stx) of Stx-producing *E. coli* (STEC) block mRNA translation by cleaving the 28S ribosomal RNA, leading to cell death⁹⁰. Subtilase (SubAB) of STEC binds N-glycolylneuraminic acid glycans and penetrates to the endoplasmic reticulum (ER), where it cleaves BiP (also known as GRP78), triggering cell death⁹⁰. **b** | Pathogenic bacteria have evolved a range of mechanisms to aid their adherence to and breaching of the endothelium and can also exploit host characteristics to this end. Membrane-anchored fibronectin-binding protein A (FnBA) of *S. aureus* bridges the bacterium to $\alpha5\beta1$ integrins ($\alpha5\beta1$) through fibronectin (FN). This leads to the formation of fibrillar adhesion-like attachment sites, bacterial movement at the surface of endothelial cells and a delay in bacterial internalization⁷⁰. Pathogen-associated molecular patterns (PAMPs) on the bacterial surface are recognized by Toll-like receptors (TLRs) on endothelial cells, leading to the initiation of a genetic programme of defence. Bacteria can traverse endothelial cells by direct transcellular invasion through macroapertures. Bacteria that target endothelial cells, such as *Rickettsia* spp. and *Coxiella* spp., have evolved strategies to invade and persist in the cells, including membrane rupture of the phagosome or formation of a parasitophorous vacuole (PV) that acquires lysosomal characteristics, respectively^{73,115}. Membrane protrusions induced by encapsulated *Neisseria meningitidis* allow the bacterium to resist blood flow forces and to destabilize cellular junctions (for detail, see main text and FIG. 3). *Bartonella henselae* produces massive membrane reorganizations. This phenomenon might favour the routing of bacteria to a compartment that is protected from lysosomal fusion⁸⁴. Rock, Rho-associated protein kinase.

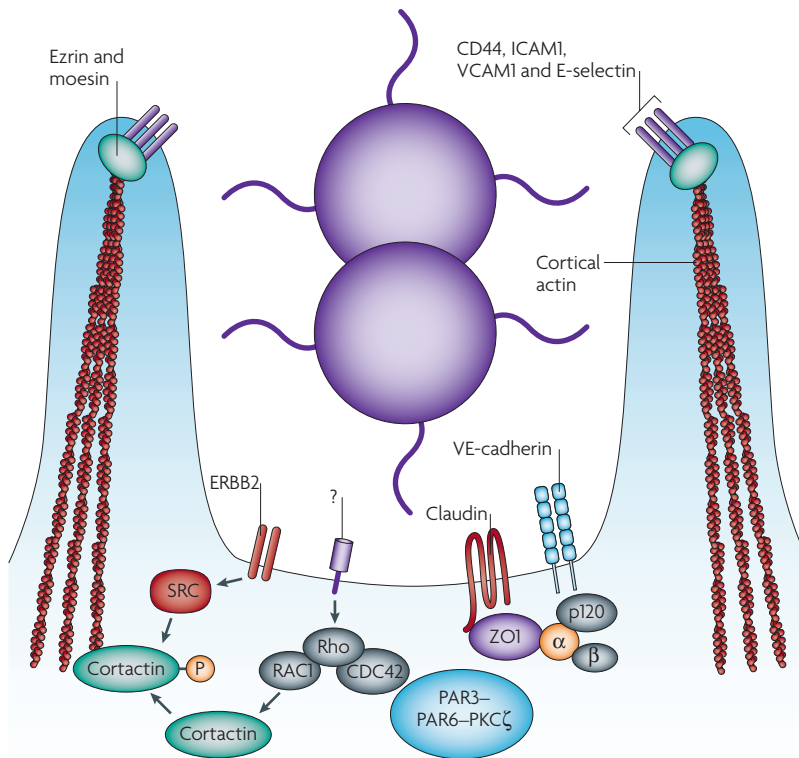


Figure 3 | *Neisseria meningitidis*. Specific molecular complexes, referred to as cortical plaques, are formed beneath adherent *Neisseria meningitidis*¹¹⁶. Ezrin and moesin, but not radixin, are recruited by *N. meningitidis* in a phosphatidylinositol-4,5-bisphosphate-dependent manner and control the organization of the cortical plaques by interacting through their amino-terminal domains with the cytoplasmic domains of transmembrane proteins such as CD44, intercellular-adhesion molecule 1 (ICAM1), vascular-cell adhesion protein 1 (VCAM1), E-selectin and F-actin^{51,53,117}. Cortical-actin polymerization, induced by *N. meningitidis*, relies on the activation of the small GTPases Rho and cell division cycle protein 42 (CDC42), along with the activation of a phosphoinositide 3-kinase (PI3K)–RAC1 GTPase signalling pathway involved in cortactin recruitment at the bacterial entry site^{51,52}. Cortactin is an actin-binding protein that is involved in the reorganization of the cortical-actin cytoskeleton, which plays a part in multiple essential cellular functions¹¹⁸. In addition, cortactin has been involved in the cellular invasion process of several bacterial pathogens¹¹⁹. The host cell tyrosine kinase receptor ERBB2 belongs to the family of epidermal growth factor receptors¹²⁰ and is activated through non-conventional formation of ligand-independent ERBB2 homodimers on the interaction of *N. meningitidis* with human endothelial cells. The clustering and activation of ERBB2 and the downstream activation of the kinase SRC induces tyrosine phosphorylation of cortactin⁵⁰. Finally, *N. meningitidis* induces the recruitment of components of the polarity complex, formed by partitioning-defective protein 3 (PAR3), PAR6 and protein kinase C ζ (PKC ζ), and components of both adherens junctions (vascular endothelial (VE) cadherin, p120 catenin (also known as δ 1 catenin) (p120), α -catenin (α) and β -catenin (β)) and tight junctions (claudin 5 and zonula occludens 1 (ZO1; also known as TJP1)) to sites of bacterial adhesion, thereby affecting the integrity of the brain endothelial junctions⁶⁰.

as fibrin traps to entangle and kill bacteria extracellularly³⁰. Induction of NETs by platelets was also observed by intravital microscopy in a model of LPS-induced sepsis. NETs retained their integrity under flow conditions and ensnared bacteria in the vasculature. However, some pathogenic bacteria, such as *Streptococcus pneumoniae*, can escape NET killing by producing endonucleases³¹. Despite the advantage gained by the host release of NETs for the destruction of bloodborne pathogens, it was found that NET release induced during septic

conditions produces collateral damage to the underlying endothelium²⁹. *In vivo* depletion of either platelets or neutrophils reduces endothelium damage. Interestingly, it has been proposed that septic patients could be treated by administering prostacyclin, a potent inhibitor of neutrophils and platelets³². Impairing the induction of NET production at the level of the endothelium might therefore be beneficial to septic patients. Furthermore, it has been shown recently that NETs may also be implicated in autoimmune vasculitis³³.

Haemostatic homeostasis. A finely tuned balance between coagulation and anticoagulation cascades maintains homeostasis of the haemostatic system. During sepsis, coagulation is amplified by the concomitant decrease of anticoagulation factors, with a reduction of thrombomodulin (TM) at the surface of endothelial cells and a reduction of circulating levels of protein C. Impaired activation of protein C favours the development of thrombosis and purpura fulminans, leading to the formation of small blood clots inside blood vessels and promoting vascular damage^{34,35}, as observed in patients with meningococcaemia, who present reduced TM levels in dermal microvessels³⁶. Activated protein C is therefore one of the few drugs to have shown some efficacy in reducing the mortality rates of patients with severe sepsis, although its positive effects can be thwarted by the associated risks of bleeding³⁷. In addition, pathogens trigger the clotting cascade through upregulation of tissue factor and activation of the extrinsic pathway (amplified by the intrinsic pathway), which ultimately leads to the generation of thrombin and fibrin. The production of fibrin stabilizes the platelet plug but has also been reported to prevent dissemination of pathogens through the blood, thus having crucial protective functions during bacterial infection³⁸. Whether coagulation is also exploited by bacteria to aid their adhesion to the endothelium is an open question. Although bacteria can activate specific coagulation factors, they generally do not initiate the coagulation cascade. Nevertheless, a recent study indicates that when *Bacillus anthracis* and *Bacillus cereus* form cell clusters, the coagulation cascade that precedes clot formation is initiated by a process known as quorum acting³⁹. Clustered *B. anthracis* cells act cooperatively to produce an operating threshold of the zinc metalloprotease immune inhibitor A (InhA1) that is sufficient to activate prothrombin and factor X directly. This mechanism does not require a change in gene expression or communication between bacteria.

Membrane adhesion and subversion

Firm bacterial adhesion to endothelial cells and the triggering of endothelial membrane reorganization are probably general requisites to circumvent the shear stress forces that are exerted by the blood flow, enabling colonization and the development of severe cardiovascular and metastatic infections⁴⁰ (see TABLE 1 and below). In addition, bacteria can also subvert cell membrane organization and receptor signalling to persist at the surface or translocate through endothelial barriers⁴¹. In a similar way, *Listeria monocytogenes* targets and crosses the

Extrinsic pathway

The primary pathway for the initiation of blood coagulation, triggered by a thrombin burst; this is also called the tissue factor pathway.

syncytiotrophoblast barrier (an epithelial tissue that replaces an endothelium at the maternal–fetal blood interface) in a manner that is dependent on the combined action of the internalins InlA and InlB, as recently shown in a humanized knock-in mouse model of listeriosis⁴². Current results suggest that adhesion to the apical surface of the syncytiotrophoblast and invasion are mediated by associations between InlA and epithelial cadherin (*E-cadherin*) as well as between InlB and MET (also known as HGF receptor). However, as InlB is only weakly attached to the bacterial surface and therefore exhibits poor adhesive properties, the prior binding of InlA and E-cadherin is required to facilitate the interaction of InlB and Met, which in turn promotes invasion. How *L. monocytogenes* targets and crosses the blood–brain barrier is currently unknown.

Adhesion of *Neisseria meningitidis* to the endothelium.

Interactions of *N. meningitidis* with human endothelia rank among the best characterized examples of complex molecular signalling pathways being hijacked by a bacterial pathogen to allow it to tightly attach at the endothelial cell surface, elicit its uptake into non-phagocytic cells and translocate through both para- and transcellular pathways (through cell junctions or by transcytosis, respectively). *N. meningitidis*, a Gram-negative bacterium, is responsible for severe sepsis and, owing to its high propensity to interact with endothelial cells and, in particular, with brain capillaries, it also triggers cerebrospinal meningitis^{41,43}. Bacterial crossing of the blood–brain barrier initiates an inflammatory response in the subarachnoid space. Because systemic bacteria are exposed to drag forces generated by the bloodstream, it has been elegantly demonstrated that the transiently slowed blood flow found in the cerebral microcirculation is responsible for the preferential attachment of *N. meningitidis* to cerebral vessels and, therefore, for the brain tropism of this pathogen⁴⁰.

Type IV pili are crucial bacterial structures that mediate the initial adhesion between capsulated meningococci and endothelial cells and promote signalling events^{44,45}. This adhesion can be further facilitated by the combined action of lipo-oligosaccharide, opacity (Opa) proteins and glycolipid adhesins⁴⁶. Binding of *N. meningitidis* type IV pili to human cells is thought to involve the cell surface receptor CD46, but this observation is not supported by all studies⁴⁷. After initial attachment, forces up to 110 pN are generated by retraction of the pili, and this is thought to be the motive force that drives the intimate association of the pathogen with the host cell surface^{48,49}. Such forces might also contribute to the resistance of *N. meningitidis* to the high velocity of the blood flow. In addition, type IV pili-dependent adhesion of *N. meningitidis* to endothelial cells promotes the local formation of membrane protrusions that are reminiscent of the epithelial microvilli structures that surround bacteria and initiate their internalization⁴⁹. The molecular events leading to the formation of these protrusions (detailed in FIG. 3) have now been well documented^{50–53}. The formation of these structures stems from the organization of specific molecular complexes involving the molecular

linkers *ezrin* and *moesin*, along with the clustering of several integral membrane proteins, including CD44, intercellular-adhesion molecule 1 (*ICAM1*) and the tyrosine kinase receptor *ERBB2* (REFS. 50,51). Activation of *ERBB2* is required to elicit efficient bacterial uptake by promoting phosphorylation of the actin-binding protein *cortactin*, which is an essential step in the control of cortical-actin assembly^{50,54}. Cortical-actin polymerization induced by *N. meningitidis* relies on the activation of both Rho GTPases and cell division cycle protein 42 (*CDC42*), along with the activation of a phosphoinositide 3-kinase (PI3K)–*RAC1* GTPase signalling pathway that is involved in cortactin recruitment at bacterial entry sites^{51,54}. Interestingly, the formation of such protrusions at sites of endothelial cell contact with meningococci was also observed *ex vivo* by transmission electron microscopy analysis of brain sections from a child who died from fulminant meningitis⁵⁵, suggesting that these structures are essential for *N. meningitidis* pathogenesis. The invagination of *N. meningitidis* in membrane protrusions enables the bacteria to resist the high mechanical forces exerted by the blood flow and to form microcolonies on the endothelial cell surface^{40,56}. Exploitation of host cell signalling pathways could therefore be pivotal for promoting intimate bacterial attachment to the cell surface, avoiding detachment under blood flow conditions. In addition, *N. meningitidis* seems to replicate in host cells, including human brain endothelial cells⁵⁷, and is capable of traversing endothelial monolayers^{51,58}. *N. meningitidis* might therefore cross the endothelial barrier by a transcytosis pathway, although few adherent meningococci are internalized and the extent to which the intracellular bacteria egress through basolateral cellular membranes is unknown. More recently, using a well-differentiated human brain endothelial cell line that exhibits blood–brain barrier characteristics⁵⁹, it has been shown that *N. meningitidis* induces the recruitment of components of the polarity complex and of adherens and tight junctions to sites of bacterial adhesion, thereby affecting the integrity of the endothelial junctions in the brain⁶⁰. The polarity complex, formed of partitioning-defective 3 (*PAR3*), *PAR6* and protein kinase C ζ (*PKC ζ*), is usually recruited at the site of initial contacts between two migrating cells, where it is needed for the recruitment of junction proteins and their subsequent formation of either adherens or tight junctions^{61,62}. Infection of brain microvessels by *N. meningitidis* therefore mimics nascent cell–cell interactions that may facilitate the exit of bacteria from the bloodstream by promoting the opening of a paracellular route. Of particular interest is the finding that the complex signalling events activated by *N. meningitidis* are similar to those induced by leukocyte adhesion on endothelial cells, which promote firm adhesion and extravasation of leukocytes through both paracellular and transcellular routes⁵³. *N. meningitidis* therefore seems to hijack the cellular machinery that is normally used for leukocyte transendothelial diapedesis to promote its own adherence to the vascular walls. In addition, by preventing the accumulation of these key proteins at leukocyte–endothelial cell contact sites, infection of endothelial cells by *N. meningitidis* prevents

Intrinsic pathway

A secondary pathway for the initiation of blood coagulation that is classically triggered by contact activation of factor XII when blood comes into contact with negatively charged surfaces (potentially collagens); this is also called the contact activation pathway.

Extravasation

The exit of leukocytes from the blood stream.

Transendothelial diapedesis

The process by which leukocytes cross the endothelium through large tunnels that are formed in endothelial cells.

leukocyte transmigration. Interestingly, the superantigen-like protein 5 of *S. aureus* was also shown to have an immunomodulatory function by binding P-selectin (which is a cell receptor that is induced by inflammation and is involved in the initial interaction between circulating leukocytes and endothelial cells), therefore preventing the initial stages of leukocyte rolling and activation^{63,64}. This might protect bacteria that are bound to endothelial cells or to the extracellular matrix from neutrophils and may block leukocyte extravasation. These results offer new, unexpected insights into how bacterial pathogens can hamper the triggering of a host inflammatory response by blocking transendothelial diapedesis of leukocytes (for a review of leukocyte diapedesis, see REF. 11,65).

Staphylococcus aureus crossing the endothelial barrier.

An unexpected example of direct crossing of the endothelial barrier comes from various clinical isolates of *S. aureus* that produce the exotoxin epidermal-cell differentiation inhibitor (EDIN)^{66,67}. EDIN factors can enter cells by macropinocytosis and, on reaching the cytosol, can inactivate the GTPase RHOA by catalysing its ADP-ribosylation at Asp-41 (REF. 18). Recent advances show that a high threshold of RHOA inhibition results in a complete disruption of actin cables and the formation of transcellular tunnels, referred to as macroapertures⁶⁸. The direct destruction of actin cables or the inhibition of Rho-associated protein kinases (Rocks) also causes macroapertures to form. The inactivation of RHOA and the formation of macroapertures do not alter the organization of adherens junctions. Strikingly, macroapertures are highly dynamic, opening and closing in minutes. During closure, actin filaments are rapidly recruited to the macroapertures, which is followed by the extension of membrane waves over the aperture (see [Supplementary information S1](#) (movie)). The opening and closing of these structures could explain the fact that EDIN triggers an increase in endothelium permeability without compromising animal viability. Macroaperture opening in rat arteries infected with EDIN-producing *S. aureus* allows bacteria to bind directly to the matrix (FIG. 2). This might represent an alternative way of crossing the endothelial barrier, as bacteria are poorly adapted to transcytosis. In line with this, *S. aureus* has evolved other strategies to avoid rapid internalization into endothelial cells. The fibronectin-binding protein A (FnbpA), an adhesion molecule of *S. aureus* that is closely homologous to Fnbp of *S. pyogenes*, allows bacterial adhesion to endothelial cells. Fnbps contain multiple modules, each of which can associate with a stretch of type I (F1) repeats of the extracellular-matrix protein fibronectin. This leaves free the well-characterized Arg-Gly-Asp motif of fibronectin that is crucial for recognition by integrins⁶⁹. Fnbps are probably able to bind to several fibronectin molecule at a time. The association of Fnbps with fibronectin allows bacteria to use fibronectin as a molecular bridge to bind to the $\alpha 5 \beta 1$ integrins of endothelial cells. This triggers the formation of fibrillar adhesion-like attachment

sites, owing to the recruitment of tensins in the absence of vinculin, paxillin and focal adhesion kinase 1 (FAK; also known as FADK1), and a specific actin reorganization that promotes bacterial movements and delays bacterial uptake⁷⁰. Such a phenomenon is thought to promote the persistence of bacteria at the cell surface for long enough to allow the pathogens to synthesize crucial amounts of cell-damaging toxins. Colonization of the endothelial surface and direct transcellular or intercellular crossing of the endothelial barrier are likely to be efficient means for bacteria, which are poorly adapted to an intracellular life style, to disseminate in tissues and form deep-seated infection foci.

The interaction of *Bartonella henselae* with the endothelium.

Intracellular colonization of endothelial cells and spreading through an intracellular route require specific virulence strategies aimed at escaping intracellular destruction by lysosomes⁷¹. For instance, obligate intracellular bacteria that target the endothelium, such as *Rickettsia* spp. or *Coxiella* spp., have evolved strategies to limit their lysosomal degradation on entry into the host cell by modulating membrane trafficking or triggering the rupture of the phagosomal membrane^{72–74}. Interestingly, a remarkable phenomenon of membrane reorganization in endothelial cells is induced by *Bartonella henselae* factors, which are injected by the bacterial type IV secretion system, and this may affect the intracellular fate of the bacteria⁷⁵. This zoonotic pathogen can induce vasoproliferative lesions in the skin and inner organs (known as bacillary angiomatosis, or bacillary peliosis in the liver) of immunocompromised patients as well as cat scratch diseases in immunocompetent individuals^{76,77}. *B. henselae* adhesion to human cells is promoted by the head domain of the major adhesin expressed by *B. henselae* biotype Marseille, *Bartonella* adhesin (BadA), the structure of which has been recently resolved^{78–80}. BadA mediates the binding of *B. henselae* to extracellular matrix proteins and to endothelial cells, possibly through $\beta 1$ integrins. Colonization and invasion of the endothelium, which occurs following a specific sequence of host-microorganism interactions, is a crucial event in the pathogenicity of this bacterium⁸¹. *B. henselae* enters human endothelial cells *in vitro* by two alternative routes: either by an endocytic uptake of individual bacteria, giving rise to *Bartonella*-containing vacuoles⁸², or by the formation of membrane waves, which form large protrusions that surround bacterial aggregates and eventually promote their internalization⁸³. These membrane protrusions, referred to as cell invasomes, are highly enriched in F-actin and tyrosine-phosphorylated proteins and exhibit a particular distribution of ICAM1 at their tips⁸³. In addition, actin cables are typically twisted around the basal part of the invasome. Interestingly, a parallel can be drawn with the adhesion of leukocytes to endothelial cells. Endothelial ICAM1 also interacts with lymphocyte function-associated protein 1 (LFA1; also known as integrin $\alpha L \beta 2$) and is enriched at the tip of the membrane projections that form membrane cups around leukocytes during their transendothelial diapedesis¹¹.

Leukocyte rolling

The method by which loosely attached leukocytes at the surface of the endothelium move.

Table 2 | Examples of bacterial toxins that target endothelial cells

Toxins	Bacteria	Biochemical activities	Cytotoxicity	Cellular targets	Cellular receptors	Refs
CDTs	Various Gram-negative bacterial strains	DNA double-strand breaks and, potentially, phosphatidylinositol-3,4,5-triphosphate 5-phosphatase	Cell cycle arrest and cell death	DNA	Various	121,122
CNF1	UPEC	Deamidase	Inflammation	Rho GTPases	67 kDa laminin receptor precursor	19,123
EDIN (types A, B and C)	<i>Staphylococcus aureus</i>	ADP ribosyltransferase	Immunoparalysis and transcellular tunnels	RHOA	Unknown	68,124
Edema toxin	<i>Bacillus anthracis</i>	Ca ²⁺ and calmodulin-dependent adenylate cyclase	Immunoparalysis	Cyclic AMP-dependent kinases	TEM8 and CMG2	103
Lethal toxin	<i>Bacillus anthracis</i>	Metalloprotease	Induction of actin stress fibres and cell death	MAPK kinases	TEM8 and CMG2	99,100,103
Lethal toxin	<i>Clostridium sordellii</i>	Glucosyltransferase	Actin disruption and apoptosis	Rac proteins, CDC42, Ras proteins and Ral proteins but not RHOA	Unknown	98,125
Stx and Stx-like	<i>Shigella dysenteriae</i> and STEC	N-glycosidase	Translation inhibition and cell death	28S ribosomal RNA	Gb3 (and Gb4 for Stx2e)	90
SubAB	STEC	Protease	Cell death	BiP (also known as GRP78)	Neu5Gc-containing glycans	93,96

CDC42, cell division cycle protein 42; CDT, cytolethal distending toxin; CMG2, capillary morphogenesis protein 2 (also known as ANTXR2); CNF1, cytotoxic necrotizing factor 1; EDIN, epidermal-cell differentiation inhibitor; Gb3, globotriaosylceramide; Gb4, globotetraosylceramide; MAPK, mitogen-activated protein kinase; Neu5Gc, glycans terminating in the sialic acid N-glycolylneuraminic acid; STEC, Shiga-like toxin-producing *Escherichia coli*; Stx, Shiga toxin; SubAB, subtilase; TEM8, tumour endothelial marker 8 (also known as ANTXR1); UPEC, uropathogenic *E. coli*.

This invasome-mediated internalization depends on the bacterial type IV secretion system VirB/VirD4 and occurs primarily by BepG-mediated inhibition of cellular endocytic processes, consequently triggering the uptake of *B. henselae* into vacuoles⁸⁴. Once internalized, bacteria can replicate in endothelial cells. These cells might be more permissive for *B. henselae* replication than macrophages owing to bacteria-triggered inhibition of the fusion of vacuole-containing bacteria with lysosomes⁸². Infection of endothelial cells, monocytes and macrophages *in vitro* results in an inhibition of host cell apoptosis and increased expression of two vasculoproliferative factors, vascular endothelial growth factor (Vegf) and hypoxia-inducible factor 1 (HIF1)^{85,86}. Recent findings suggest that BepA, a potent anti-apoptotic translocated virulence factor, promotes the formation of typical capillary-like sprouts in infected endothelial cells^{75,87}. However, the causal role of the type IV secretion system and its effectors in bacillary angiomatosis and bacillary peliosis remains elusive, owing to the lack of a suitable animal model.

Toxin-induced endothelial damage

Recent advances highlight the fact that direct damage to endothelial cells caused by bacterial toxins has a major impact on the induction of haemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpura, intravascular coagulation and other vascular dysfunctions in target tissues.

The endothelium and epithelium of the renal vasculature, especially that of glomeruli, are targets for the toxins that are produced by various *Escherichia coli* O157:H7 or non-O157 toxigenic strains, referred as Shiga-like toxin-producing *E. coli* (STEC)^{88,89} (TABLE 2). Ruminants are the main reservoir of STEC, which is transmitted to humans primarily through contaminated foods and water. Clinical manifestations caused by STEC range from mild, watery diarrhoea to haemorrhagic colitis, HUS and death. These bacterial toxins probably transcytose through the gut epithelium, disseminate haematogenously and intoxicate the endothelial cells of the kidney and brain. The intoxication, swelling and detachment of endothelial cells impairs their anticoagulation activity and unmasks the endothelium basement membrane, initiating the local deposition of fibrin-platelet thrombi and the influx of inflammatory cells. The contribution of each of the various toxins found in STEC isolates during colonization, disruption of the gastrointestinal barrier and induction of HUS remains to be fully determined (TABLE 2). The Shiga toxin and Shiga-like toxins comprise a polypeptide A subunit bearing the cytotoxic activity, which is non-covalently associated to five cell receptor-binding B fragments that make up the B subunit (AB₅)⁹⁰. The cooperative binding of the B fragments to the glycosphingolipid globotriaosylceramide (Gb3) can deform the plasma membrane at high toxin concentrations to induce membrane invagination and toxin entry⁹¹. The A subunit has

N-glycosidase activity, which cleaves the 28S rRNA of the 60S ribosomal subunit, leading to the inhibition of protein synthesis and the induction of cell death. Following the binding of Shiga toxins to the surface of human glomerular microvascular endothelial cells (GMVECs) and other cell types, the AB₅ complex is conveyed to the endoplasmic reticulum (ER) by retrograde transport, and the A subunit is then transported to the cytosol⁹². Interestingly, the production of cytokines, in particular tumour necrosis factor (TNF) and interleukin-1 β , by intoxicated monocytes can upregulate the expression of Gb3 and therefore sensitizes GMVECs to Shiga toxins⁹². STEC isolates are a very diverse group of pathogenic *E. coli*⁹³. Although Shiga toxins are key virulence factors in HUS, epidemiological studies indicate that other factors, such as the subtilase (SubAB) cytotoxin, confer higher virulence properties to STEC for human infections^{93,94}. SubAB cleaves an essential ER chaperone, BiP (also known as GRP78), which activates various ER stress-signalling pathways and causes HUS in mice^{93,95}. Like Shiga toxins, SubAB is an AB₅ toxin. It has been recently discovered that the binding subunit, SubB, has a strong preference for glycans terminating in the sialic acid *N*-glycolylneuraminic acid (Neu5Gc), which is a monosaccharide that is not synthesized in humans owing to the non-functionality of the cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase gene, *CMAH*⁹⁶. Nevertheless, this monosaccharide is abundant in lamb, pork, beef and dairy products, possibly as a result of contamination by STEC. Dietary Neu5Gc can be assimilated by humans, creating high-affinity SubB receptors on gut epithelia and kidney vasculature and leading to a sensitization of the host to the deadly action of this toxin⁹⁶.

Bacteria-induced endothelial dysfunctions can also result from toxin targeting of essential host cell signalling molecules that are involved in actin cytoskeleton reorganization. Indeed, pleural effusion and toxic shock syndrome induced by *Clostridium sordellii* results from the lethal toxin-induced destruction of the endothelial-cell actin cytoskeleton through the inhibition of various small GTPases, including the Rac proteins^{97,98}. Recent advances have shown that the lethal toxin (LT) of *B. anthracis* has a potent inhibitory activity on MAP kinase signalling that not only paralyzes host defences, but also directly triggers cytotoxicity in human endothelial cells, as explained below^{99–101}. Interestingly, LT-mediated cytotoxicity could be useful to treat cancer angiogenesis¹⁰². Vascular leakage, pleural effusion and haemorrhage are hallmarks of systemic infection by the Gram-positive, spore-forming *B. anthracis*. A major virulence factor of *B. anthracis* is its ability to synthesize two toxins from different combinations of three polypeptides: the protective antigen (PA), the edema factor (EF) and the lethal factor (LF)¹⁰³. Heptamers of PA can associate with either LF or EF to form LT (made of PA and LF) or the edema toxin (made of PA and EF). Two receptors for PA, each containing a von Willebrand factor A-like domain, have been characterized: tumour endothelial marker 8

(TEM8; also known as ANT XR1) and capillary morphogenesis protein 2 (CMG2; also known as ANT XR2)^{104,105}. TEM8 is highly expressed in epithelial cells lining *B. anthracis* entry sites and tumour vasculature, whereas CMG2 is expressed in most human cells^{106,107}. Heptamers of PA escort LF and EF into host cell endosomes and promote their injection into the cytosol^{108,109}. When in the cytosol, LF uncouples MAP kinase signalling cascades by cleaving the amino-terminal part of MEKs (MAP kinases/extracellular signal-regulated kinases)¹¹⁰. Intriguingly, the vascular dysfunctions that are associated with pleural effusion and hypoxia induced by LT are independent of TNF production¹¹¹. A recent advance in our understanding of how LT triggers deadly vascular leakages comes from the observations that LT induces actin cytoskeleton reorganization and apoptosis in human endothelial cells *in vitro*^{99–101}. Indeed, inhibition of MEKs by LT triggers the formation of actin cables and, consequently, a disorganization of the endothelial intercellular junctions, which probably plays a key part in the induction of endothelial permeability^{100,112}. The molecular mechanisms leading to this actin cable formation and to apoptosis induction, as well as the contribution of both phenomena in LT-induced vascular leakages, await new studies. A large number of microbial virulence factors target Rho and MAP kinase signalling^{18,113}. Their possible contribution to endothelium dysfunctions remains to be investigated.

Perspectives

Cellular microbiology, combined with genetics and biophysics approaches, has been tremendously efficient in shaping our current vision of many of the key molecular steps involved in endothelium infection by bloodborne bacteria.

Studies using differentiated primary cells have revealed a high level of complexity in the interplay between pathogens and both the host cell membrane and cell–cell junctions, as well as in pathogen effects on host gene expression. With the discovery of many species-specific receptor–ligand interactions between host and pathogen, it has become possible to generate humanized animal models of infection, as recently demonstrated for *L. monocytogenes* maternal–fetal dissemination. The integration of additional approaches such as epidemiology, bacterial whole-genome sequence comparisons and intravital imaging now allows us to define, with precision, the spatio-temporal features of microbial infections. A better knowledge of host–pathogen interactions will hopefully allow the development of effective countermeasures that interfere with the initial steps of pathogen interactions with the host. For instance, a detailed characterization of *N. meningitidis* receptor complexes at the blood–brain barrier will contribute to a better understanding of *N. meningitidis* dissemination to the brain. In addition, one can now also envision the hijacking of potent bacterial effectors to generate recombinant toxins for use as new therapeutics, for example to counteract cancer neo-angiogenesis.

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Competing interests statement

The authors declare no competing financial interests.

DATABASES

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