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Bacteria and Endothelial Cells: A Toxic Relationship

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Abstract

Pathogenic bacteria use the bloodstream as a highway for getting around the body, and thus have to find ways to enter and exit through the endothelium. Many bacteria approach this problem by producing toxins that can breach the endothelial barrier through diverse creative mechanisms, including directly killing endothelial cells (ECs), weakening the cytoskeleton within ECs, and breaking the junctions between ECs. Toxins can also modulate the immune response by influencing endothelial biology, and can modulate endothelial function by influencing the response of leukocytes. Understanding these interactions, in both the *in vitro* and *in vivo* contexts, is of critical importance for designing new therapies for sepsis and other severe bacterial diseases.

Introduction

Every successful bacterial pathogen must at some point interact with the body's largest organ –the endothelium. This interaction can take many forms (Fig 1). Bacteria can disrupt the barrier function of the endothelium to gain access to the bloodstream and then again to gain access to new organs. Many bacteria have evolved toxins which can increase vascular permeability by either killing ECs or by interfering with the cytoskeleton or cell-cell junctions. This review will focus on some recent findings elucidating the activities of toxins and other secreted effectors that bacteria use to attack the endothelium (Table 1).

Toxins that disrupt endothelial barrier function directly

Toxins from gram-positive bacteria

Many gram-positive bacteria produce toxins that directly kill ECs, often by forming pores in the plasma membrane of host cells. Pneumolysin (Ply), a cholesterol-dependent cytolysin secreted by *Streptococcus pneumoniae*, is an early-discovered example of a toxin that targets ECs [1,2]. Ply kills ECs through apoptosis in a manner dependent on the activation of p38 MAPK [3]. Beyond simply killing ECs, Ply can also manipulate their biology in other ways. The pores formed by Ply in the plasma membrane of ECs cause calcium flux activating phospholipase A2, which has the potential to produce inflammatory mediators [4]. Further, through the activation of protein kinase C- α , Ply can increase endothelial permeability by

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perturbing the microtubule cytoskeleton, decreasing vascular endothelial (VE)-cadherin expression, and increasing production of arginase, which further impairs endothelial barrier function by reducing the production of NO [5].

Staphylococcus aureus also targets ECs, via α -toxin (also known as Hla) [6]. As with Ply, α -toxin can also have effects beyond direct killing, as it can impair the endothelial barrier by causing its receptor, ADAM10 [7] to cleave VE-Cadherin [8], disrupting cell-cell junctions and contributing to sepsis severity [8]. Junctional tropism appears to be key to α -toxin action, as PLEKHA7, a component of adherens junctions, is necessary for α -toxin to be able to kill epithelial cells [9]. Since ECs also express PLEKHA7, this host factor likely plays a similar role in their susceptibility to *S. aureus* α -toxin.

Lastly, *Bacillus cereus* produces several enterotoxins, including the three-component toxins non-hemolytic enterotoxin (Nhe) and hemolysin BL (HBL) that can also injure ECs. Based on *in vitro* experiments, ECs are highly susceptible to Nhe, and to a lesser extent to HBL. Further, while HBL kills rapidly by pore formation, Nhe kills more slowly, through mitochondrial damage [10]. This study investigated the cytotoxicity of these three toxins on different cell types, and found marked differences in susceptibilities, strongly suggesting the existence of specific receptors for these toxins.

Toxins from gram-negative bacteria

Many gram-negative bacteria are also adept at secreting toxins that disrupt endothelial integrity, through both cytolytic and non-cytolytic mechanisms. One of the best examples is *Pseudomonas aeruginosa*, which produces both type 2 and type 3 secretions systems (T2SS and T3SS). These systems secrete effectors that disrupt endothelial barrier integrity, causing devastating pathology in the lung. The T3SS can secrete exoenzymes S, T, U and Y. ExoU directly lyses ECs through its phospholipase activity, and this is partly achieved through the induction of oxidative stress [11,12]. ExoU can also induce a pro-thrombotic state, by causing ECs to release von Willebrand Factor (vWF) and vWF-expressing microparticles, resulting in increased platelet adhesion and disrupting vascular flow [13].

ExoS and ExoT are highly related toxins that cause leakage across the lung endothelium by manipulating the activities of Rho GTPases, leading to the formation of actin stress fibers [14]. The inhibition of Rho GTPases eventually leads to the dephosphorylation of Lim kinase, inducing the actin-severing activity of cofilin, finally leading to cell retraction [15]. Other studies, however, have found that the endothelial disruption of the *P. aeruginosa* T3SS is primarily mediated through ExoY, an adenylyl cyclase which causes microtubule breakdown, resulting in gaps between ECs and tissue edema [16]. Further, ExoY prevents repair of the endothelium by decreasing the replication of ECs, even several days after exposure. This potentially occurs because of prolonged phosphorylation of tau and the formation of tau aggregates [17]. To further complicate this picture, LasB, secreted through the T2SS, was recently found to cleave VE-Cadherin, further facilitating the disruption of endothelial monolayers. Epithelial-cadherin, however, was not affected, indicating the specificity of this toxin's action [18]. Altogether, these findings demonstrate that *P. aeruginosa* is most certainly adept at destroying the barrier integrity of the endothelium.

There are distantly related isolates of *P. aeruginosa* that lack the T3SS, and yet are highly virulent. One representative of such isolates is strain CLJ1, which was isolated from a patient with hemorrhagic pneumonia [19]. This strain kills ECs by producing a novel toxin known as Exolysin (ExlA) [19]. ExlA is highly cytotoxic to ECs and increases lethality in a mouse pneumonia model [19]. Other isolates producing ExlA have been identified, and are toxic to ECs as well [20].

Toxins that affect the immune functions of endothelial cells

The endothelium also performs important immune functions. ECs sense microbes through toll like receptor (TLR) stimulation and other innate sensing mechanisms, becoming activated to secrete cytokines and chemokines and upregulate leukocyte adhesion molecules to direct leukocyte traffic to the infected area [21]. Many bacteria manipulate this response. For instance, Shiga-toxigenic *Escherichia coli* produces subtilase cytotoxin (SubAB), an AB5 cytotoxin, that cleaves BiP, an ER chaperone [22].

In several cells lines including ECs, this decreases the secreted levels of IL-8, MCP-1 and IL-6 post-translationally, seemingly by inducing ER stress and the unfolded protein response [23]. In contrast, Shiga toxin 2 (Stx2), another AB5 toxin, upregulates cytokine and chemokine expression [23]. This study found that the combination of SubAB and Stx2 inhibited the secretion of IL-8 from monocytic U937 cells, but did not test the combination in ECs. Thus, these two toxins together may either strengthen or dampen the secretion of cytokines from ECs, or potentially both in different situations.

P. aeruginosa ExoU can also modulate the leukocyte-directing functions of ECs. Through its phospholipase activity, ExoU causes a release of eicosanoids from ECs, which causes inflammation in mouse footpad and lung infection models [24]. ExoU also cleaves intracellular adhesion molecule-1 (ICAM-1), releasing it from the membrane. Soluble ICAM-1 increases inflammation by activating leukocytes, contributing to pathogenesis [25].

Autophagy: Endothelial cells fight back

There are two recent examples in the literature which illustrate that ECs are not completely defenseless against the toxins that bacteria attack them with, both involving autophagy. Maurer *et. al.* made the surprising observation that mice defective in autophagy are more susceptible to intravenous and lung infection with *S. aureus* strains that produce high amounts of α -toxin. Further, deficiency of autophagy in ECs is sufficient to render mice more susceptible to α -toxin. Autophagy protects ECs from α -toxin by decreasing the levels of α -toxin's receptor, ADAM10, in a post-transcriptional manner [26]. This study illustrates how much we can learn from studying host-pathogen interactions *in vivo* and argues that *in vivo* studies are required to complement *in vitro* cell biology to fully uncover how pathogens interact with ECs and other host cells and organs.

In another example, intracellular Group B *Streptococcus* β -hemolysin/cytolysin (β -h/c) triggers autophagy in ECs, which in turn kills the majority of the internalized bacteria [27]. Thus, in both of these examples ECs are able to fight back with autophagy, but not effectively enough to completely prevent disease. Presumably there are examples of less

successful pathogens that ECs are able to withstand through autophagy and other yet to be described resilience mechanisms. However, it is fair to assume that pathogens have developed clever ways to subvert ECs and their resilience mechanisms to promote infection and/or their survival within the mammalian host.

Outer Membrane Vesicles

Gram-negative bacteria release outer membrane vesicles (OMVs), another type of secreted effector. OMVs turn endotoxin and other surface-associated virulence factors into exotoxins, so that the inflammatory stimulus of localized bacteria is felt systemically. OMVs from *E. coli* cause increased ICAM-1, E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and IL-6 expression in ECs *in vitro* and *in vivo*. These effects are dependent on TLR4 and NF κ B and are more profound than stimulation by free LPS [28,29]. Further, *E. coli* can use OMVs as another mechanism to secrete toxins. For instance, enterohemorrhagic *E. coli* (EHEC) produces EHEC-hemolysin (EHEC-Hly), a member of the repeats-in toxin family, which it can secrete free or OMV-associated. Free EHEC-Hly lyses ECs. OMV-associated EHEC-Hly, however, is endocytosed by ECs, escapes into the cytoplasm, and forms pores in mitochondria, which ultimately leads to apoptosis [30].

Gram-positive bacteria can also release membrane vesicles (MVs) which can contribute to pathogenesis. *S. pneumoniae*, for example, releases MVs which are high in lipoproteins and contain pneumolysin [31]. The impact of OMVs and MVs on the endothelium is an interesting area for future research.

Effects on the vasculature: beyond endothelial cells

Bacterial toxins can also manipulate components of the vasculature other than ECs. *Bacillus anthracis* produces lethal toxin (LT), a metalloproteinase, and edema toxin (ET), an adenylate cyclase. LT and ET can both induce vascular shock in mice, which was thought to be mediated by their effects on the endothelium [32,33]. However, analysis of mice with various conditional deletions of capillary morphogenesis protein-2 (CMG2), a critical anthrax toxin receptor [34,35], clearly showed that LT kills by targeting cardiomyocytes and vascular smooth muscle, while ET kills by targeting hepatocytes, but endothelial targeting is not required for either process [36].

Vascular leakage can also be induced indirectly. *S. aureus* phenol-soluble modulins α 4 (PSM α 4) has recently been shown to stimulate heparin-binding protein release from neutrophils, which subsequently increases endothelial permeability [37]. Remarkably, an isogenic *S. aureus* mutant for PSM α 4 caused less vascular leakage in mouse lung tissue than WT in an intravenous model of infection, even though this mutant still had α -toxin, which directly kills murine ECs.

These two examples illustrate the many ways that bacterial toxins can manipulate the vasculature without directly targeting ECs. Moreover, they demonstrate that the ability of toxins to mediate phenotypes *in vitro* does not guarantee that these phenotypes are important for pathogenesis of a given type of infection *in vivo*.

Conclusion

Pathogenic bacteria are clearly very adept at damaging and manipulating the endothelium in order to disseminate and cause severe disease. In response, we must be adept at developing ways to protect the endothelium from these attacks. While bacteria and toxin specific therapies are important, we should not lose sight of more generalized methods for protecting the vasculature. For instance, recently it has become increasingly clear that statins and angiotensin receptor blockers exert protective effects on the vasculature during infection [38–40]. Though several studies have proposed mechanisms of how these drugs protect ECs by strengthening them in a general manner [41–43], they may in some cases also act by protecting ECs from the actions of toxins, as has been shown for Ply in a mouse model of sickle cell disease [44]. Additional research into the effects of these and other drugs on the endothelium, bacteria, and bacterial toxins could be important as they could uncover novel strategies to protect the endothelium from infectious insults.

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Highlights

1. Endothelial targeting is an important part of the pathogenesis of many bacteria.
2. Many gram-positive bacteria such as *S. pneumoniae*, *S. aureus*, and *B. cereus* kill endothelial cells by releasing pore-forming toxins.
3. Gram-negative bacteria such as *P. aeruginosa* compromise the barrier function of the endothelium by secreting toxins that disrupt the cytoskeleton and cell-cell interactions. There are examples of cytolytic toxins that gram-negative bacteria secrete as well.
4. Bacterial toxins can also influence the immune response by modulating the endothelial recruitment of immune cells.
5. Membrane vesicles are another example of a secreted effector that bacteria can use to target endothelial and other host cells.

Figure 1.

The many ways bacteria can interact with ECs. Bacteria can adhere to ECs, and use type 3 or 4 secretion systems to inject toxins. Bacteria can secrete toxins into the bloodstream which then bind to ECs (in some cases through a receptor) and kill the cells. OMVs can also be used to deliver toxins to ECs. Toxins can also manipulate cells to disrupt their cytoskeleton and their contacts with other ECs. Toxins and OMVs can modulate the inflammatory state of ECs, affecting leukocyte adhesion molecules and cytokines, to increase or decrease leukocyte migration to the area. The end results of these actions can include bacterial breach of the endothelium, leakage of plasma and edema, and increased or decreased leukocyte migration and leukocyte-mediated damage in infected tissues.

Table 1

Some Recent Findings in Bacterial Toxin/ Endothelial Cell Interactions

Bacterium	Toxin	Models studied	Recent finding	Ref
Gram-positive				
<i>Streptococcus Pneumoniae</i>	Pneumolysin	Human umbilical vein ECs (HUVECs)	Induces apoptosis through p38 MAPK activation	[3]
		Human lung microvascular ECs (HL-MVECs), intratracheal intoxication of mice	Disrupts barrier function through PKC α	[5]
<i>Staphylococcus aureus</i>	α -toxin	Intravenous and intradermal mouse intoxication, Human pulmonary artery ECs (HPAECs)	Induces loss of endothelial barrier function	[7]
		Haploid human cells (HAP1), intranasal and skin mouse infection	Requires PLEKHA7 for epithelial cell killing	[9]
<i>Bacillus cereus</i>	Nhe, HBL	HUVECs	Kill ECs, particularly Nhe	[10]
Gram-negative				
<i>Pseudomonas aeruginosa</i>	ExoU	Human microvascular endothelial cell line-1 (HMEC-1)	Induces a pro-thrombotic state	[13]
		HMEC-1	Cleaves ICAM-1 from the membrane	[25]
	ExoS, ExoT	HUVECs, Intranasal mouse infection	Breaks down actin filaments through cofilin	[15]
	ExoY	Pulmonary microvascular ECs (PMVECs)	Impairs EC repair	[17]
	LasB	HUVECs, Intranasal neutropenic mouse infection	Cleaves VE-Cadherin	[18]
	Exolysin	HUVECs, Intranasal mouse infection	Kills ECs	[19]
Shiga-toxigenic <i>Escherichia coli</i>	SubAB	HBMECs	Decreases cytokine secretion	[23]
	Shiga toxin 2	HBMECs	Increases cytokine and chemokine expression	[23]