

Intracellular bacteria and endothelial cytokine release with

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How many intracellular bacteria can effect endothelial cells?

Stefan Hippenstiel: There are “classical” intracellular bacteria and a lot of facultative intracellular bacteria, which invade and possibly replicate inside of endothelial cells. *Rickettsiae*, *Bartonella henselae* and *Chlamydia pneumoniae* are members of the first group. *Listeria monocytogenes* is a typical example of a facultative intracellular bacterium capable of invading and replicating inside of the endothelium. However,

there are a lot of bacteria, which transiently invade endothelial cells like *Streptococcus pneumoniae*, *Neisseria meningitidis*, or *Staphylococcus aureus*, but there is less knowledge what happens during this interaction. In principle, all invasive pathogens entering tissue or the bloodstream get in close contact with the endothelium and to date for most of these pathogens invasion of endothelial cells is not investigated (1).

What is the result of an infection?

Stefan Hippenstiel: In most cases acute infection with intracellular bacteria induces a pro-inflammatory endothelial phenotype. This includes the release of various chemokines, vasoactive factors as well as loss of the anticoagulant properties of the endothelium. Increased expression of adhesion molecules on endothelial cell surface after infection with e.g. *C. pneumoniae* (2) or *L. monocytogenes* (3) allows recruitment of leucocytes. Under some circumstances, intracellular bacteria may induce (e.g. *S. aureus*) or inhibit (e.g. *C. pneumoniae*) endothelial apoptosis (1).

Barthoneilla causing e.g. bacillary angiomatosis-peliosis and cat-scratch disease is the only bacterial pathogen known to induce angioproliferation. A fascinating hypothesis has been proposed that *Agrobacterium tumefaciens* and *Barthoneilla* share the same strategy to survive by triggering the promotion of their own habitat (4). Infections with e.g. *Rickettsiae* may result in potential lethal disease like Rocky Mountain spotted fever or Mediterranean spotted fever. If chronic endothelial infections by *C. pneumoniae* in humans contribute to the development of atherosclerosis is still unclear.

Which cytokines can be released from endothelial cells?

Stefan Hippenstiel: In general, well-known pro-inflammatory cytokines like IL-8, IL-6, or chemokines like IL-1 β or TNF α could be released by human endothelial cells. It is of interest to note that nearly all experiments regarding

endothelial activation induced by bacteria are conducted by using endothelial cells cultured under static conditions. In endothelium cultured under flow strong up-regulation of the transcription factor Krüppel-like factor-2 (KLF-2)

was documented. Importantly, SenBanerjee et al demonstrated that shear stress inhibited IL-1 β - or endotoxin-induced E-selectin expression in a KLF-2 dependent manner (5). Thus, although bacteria-infected

endothelial cells *in vitro* liberated a broad array of chemokines, future studies should be performed with endothelial cells exposed to flow to take advantage of this awareness.

Which type of signal pathways are involved?

Stefan Hippenstiel: Endothelium-invading pathogens are first recognized by membrane bound pathogen recognition receptors (PRR) (e.g. TLRs). This leads to the activation of TLR-typical downstream signalling pathways including p38, ERK, and JNK MAP kinase as well as the transcription factor NF- κ B (1). In the case of intracellular bacteria, cytosolic PRRs may also contribute to endothelial activation. Nucleotide-binding oligomerization domain (Nod) proteins are considered as such cytosolic PRRs. We found that in endothelial cells intracellular *C. pneumoniae* were recognized by Nod1 whereas extracellular *Chlamydia* were detected by TLR2 (6). In addition, Nod1 is critically involved in chemokine secretion, NF- κ B and p38 activation initiated by *L. monocytogenes* in endothelial cells. Thus, membrane bound as well as cytosolic receptors contribute to the inflammation

process induced by intracellular bacteria in endothelial cells. Recently type I interferons were suggested to participate in host cell response against intracellular bacteria. Interestingly, IFN β induction by *Listeria* was independent of the TLRs, Rip2, and Nod1/2. Thus, so far unidentified receptors might be involved in the IFN β induction induced by *Listeria* (and possibly other bacteria). Besides recognition of bacteria by host PRRs, bacteria may hijack host-signaling pathways thereby manipulating the host behaviour. E.g. *Listerial* phospholipases stimulate endothelial ceramide production thereby activating the NF- κ B pathway (3) while *Chlamydia* acquire target cell sphingolipids essential for their intracellular replication. However, there are only few studies about this topic, which only may show the tip of the iceberg.

Any relevance to novel therapeutical approaches?

Stefan Hippenstiel: In general, analysis of molecular mechanisms of pathogen-host interaction is an interesting way to give a rational basis for the development of new therapeutic interventions beyond antibiotic treatment. It seems reasonable to suggest that e.g. inhibition of cell sphingolipid supply for *Chlamydia* by endothelial cells may reduce colonisation of the endothelium. Moreover, an intervention which blocks bacteria adhesion to and uptake in endothelial cells is also a promising way to reduce host susceptibility. For example, in staphylococci disease, manipulation of “secretable expanded repertoire

adhesive molecules” (SERAM) like Efb, Emp, or Eap may pave the way to new therapeutic interventions (7). Overall, the field of endothelial-specific cellular microbiology is still at its beginning and therefore the potential therapeutic role of involved signalling molecules or cytosolic PRRs needs further investigation.

REFERENCES

- Hippenstiel S et al. Thromb Haemost 89, 18, 2003
- Krüll M et al. J Immunol 162, 4834, 1999
- Schwarzer N et al. J Immunol 161, 3010, 1998
- Kempf VA et al. Trends Microbiol 10, 269, 2002
- SenBanerjee S et al. J Exp Med 199, 1305, 2004
- Opitz B et al. Circ Res 96, 319, 2005
- Chavakis T et al. Thromb Haemost 94, 278, 2005