Long noncoding RNAs in bacterial infection

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Abstract
Infectious and inflammatory diseases remain major causes of mortality and morbidity worldwide. To combat bacterial infections, the mammalian immune system employs a myriad of regulators, which secure the effective initiation of inflammatory responses while preventing pathologies due to overshooting immunity. Recently, the human genome has been shown to be pervasively transcribed and to generate thousands of still poorly characterized long non-coding RNAs (lncRNAs). A growing body of literature suggests that lncRNAs play important roles in the regulatory circuitries controlling innate and adaptive immune responses to bacterial pathogens. This review provides an overview of the roles of lncRNAs in the interaction of human and rodent host cells with bacterial pathogens. Further decoding of the lncRNA networks that underlie pathological inflammation and immune subversion could provide new insights into the host cell mechanisms and microbial strategies that determine the outcome of bacterial infections.

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1 | INTRODUCTION

The human skin and mucosal surfaces closely interact with bacterial communities, which colonize the host as symbionts and contribute to homeostasis. During bacteria–host co-evolution several microbial species have developed strategies to overcome these barriers and manifest an infection, for example, by altering phagosomal maturation, cell death and immune response pathways (Bengoechea & Sa Pessoa, 2019; Rocco & Irani, 2011; Sindhwani & Arya, 2017). By combating such facultative or obligate pathogenic bacteria, the cellular immune system plays a key role in the maintenance of organismal homeostasis. Whereas the innate immune system confers immediate defense against invading pathogens, long lasting protection is provided by the adaptive immune system.

Different from adaptive immune cells, which sense foreign antigens by a nearly infinite arsenal of B- and T-cell receptors (BCRs, TCRs), generated by somatic DNA recombination, the innate immune system relies on germ-line
encoded pattern recognition receptors (PRRs). Several PRRs are deeply conserved throughout bilaterian animal evolution and sense equally conserved pathogen structures, referred to as pathogen associated molecular patterns (PAMPs) (Akira et al., 2006), as well as damage associated molecular patterns (DAMPs), released by damaged tissue and necrotic cells (Rathinam & Fitzgerald, 2016; Takeuchi & Akira, 2010). A prototypical example is the plasma membrane standing Toll-like receptor 4 (TLR4), a member of a PRR family originally identified in fruit flies (Lemaitre et al., 1996). Mammalian TLR4 senses extracellular bacterial cell wall lipopolysaccharide (LPS) and activates two distinct intracellular signaling programs (Figure 1). Upon activation of TLR4, the intracellular adapter Myeloid Differentiation Primary Response Protein 88 (MyD88) forms a complex with Interleukin-1 Receptor Associated Kinase 1 (IRAK1) (Lin et al., 2010). This results in auto-phosphorylation of IRAK1 and its dissociation from MyD88 (Jiang et al., 2002). IRAK1 subsequently promotes TNF Receptor Associated Factor 6 (TRAF6) auto-ubiquitination and Transforming Growth Factor-β-activated Kinase 1 (TAK1) ubiquitination. TAK1 in turn forms a complex with the TGF-Beta Activated Kinase 1 Binding Proteins TAB1, TAB2, and TAB3 (Z. J. Chen, 2012) to trigger C-Jun N-Terminal Kinase (JNK)/p38 Mitogen-Activated Protein Kinase (MAPK) dependent activation of the transcription factor Activator Protein 1 (AP1) or IKK complex dependent activation of the Nuclear Factor Kappa B (NFkB) transcription factor (Akira et al., 2006; Takeuchi & Akira, 2010). Upon binding of TAK1 to the IKK complex via ubiquitin chains, Inhibitor Of NFkB Kinase Beta (IKKβ) is phosphorylated and activated. This triggers phosphorylation, ubiquitination and subsequent degradation of the cytoplasmic NFkB inhibitor IκBα, resulting in nuclear translocation of NFkB and activation of the majority of LPS-induced pro-inflammatory immune genes (Akira et al., 2006; Takeuchi & Akira, 2010) (Figure 1). In addition to MyD88, TLR4 can also signal through the TIR Domain-Containing Adapter Protein Inducing IFN-Beta (TRIF), which interacts with TNF Receptor Associated Factor 3 (TRAF3). Upon auto-ubiquitination, TRAF3 promotes phosphorylation of TANK Binding Kinase 1 (TBK1), which in turn phosphorylates the Interferon Regulatory Factor 3 (IRF3) transcription factor (Akira et al., 2006). Upon nuclear translocation, IRF3 induces transcription of type I interferon (IFN) genes (Akira et al., 2006). Upon release, type I IFN binding to IFN

**FIGURE 1** Major bacterial recognition pathways in the immune system. TLRs, such as TLR4 can activate NFkB and IRF3 dependent inflammatory mediator and type I IFN production through the MyD88 and TRIF pathway, respectively. NLRs can activate NFkB (e.g., NOD2) or inflammasome-dependent cytokine maturation (e.g., NLRC4). RLRs, such as RIG1 promote type I IFN production through the MAVS pathway. Type I IFN stimulates STAT-dependant interferon induced gene (ISG) expression via the IFNAR1/2 receptor.
alpha receptor (IFNAR) dimers triggers Janus Kinase (JAK) transphosphorylation and JAK-dependent Signal Transducer And Activator Of Transcription (STAT) phosphorylation (Rawlings et al., 2004) (Figure 1). Activated STAT proteins, such as STAT1, promote the transcription of interferon-stimulated genes (ISGs) in a wide range of target cell types (Figure 1) (Sviecki & Colonna, 2011). Proteins encoded by ISGs in turn induce an infection-refractory state, for example, through inactivation of viral transcription and translation pathways (Kagan & Barton, 2014; Schneider et al., 2014; Takeuchi & Akira, 2010; Trinchieri, 2010). Besides interference with viral replication, type I IFNs are increasingly recognized to promote defense against bacterial pathogens (Boxx & Cheng, 2016). Type I IFN responses, can, however, also contribute to infection-induced pathologies and for instance exacerbate pulmonary tuberculosis or increase susceptibility to Listeria monocytogenes infection (Stifter & Feng, 2015). TLR4 is the only PRR capable of inducing both the MyD88- and the TRIF-dependent immune signaling pathway (Takeuchi & Akira, 2010). The human TLRs signaling through MyD88 are the flaggellin sensor TLR5, the single-stranded RNA (ssRNA) sensors TLR7 and 8, the CpG DNA receptor TLR9 and the lipoprotein sensor TLR2, which acts in conjunction with TLR1 or 6, TLR3, which senses double-stranded RNA (dsRNA), signals through the TRIF pathway (Takeuchi & Akira, 2010). Other PRR families are the C-type lectin receptors (CLRs), the RigI-like receptors (RLRs) and the Nucleotide-binding Oligomerization Domain (NOD)-like receptor (NLR) family. While the CLRs primarily participate in antifungal defense, RLRs sense viral and bacterial nucleic acids and induce type I IFN expression through the Mitochondrial Antiviral Signaling Protein (MAVS), instead of the TRIF signaling adapter (Takeuchi & Akira, 2010). The NLR family members NOD1 and NOD2 sense intracellular bacterial peptidoglycans and activate NFκB and AP1 through a signaling cascade depending on Receptor Interacting Serine/Threonine Kinase 2 (RIP2) instead of MyD88 (Takeuchi & Akira, 2010). Other members of the NLR family are engaged in the inflammasome pathways, which are activated by cytosolic DAMPs and PAMPs. While NLR Family Pyrin Domain Containing 3 (NLRP3) for example seems to promote inflammasome activation upon diverse DAMP signals, such as excess ATP, NLR Family CARD Domain Containing 4 (NLRC4) senses bacterial type 3 secretion system components and intracellular flaggellin (Lamkanfi & Dixit, 2014) (Figure 1). Cytosolic activation and oligomerization of inflammasome associated NLRs promotes Apoptosis-Associated Speck-Like Protein Containing A CARD (ASC) dependent caspase-1 activation, resulting in proteolytic maturation of the cytokines IL1β and IL18 (Rathinam & Fitzgerald, 2016). Caspase-1 furthermore promotes pyroptosis, a rapid form of cell death, exposing cytoplasmic content, including intracellular pathogens, to phagocytes in the periphery (Jorgensen & Miao, 2015). Thus, pyroptosis is fundamentally different from classic, caspase-3/7 induced apoptosis downstream of Cytochrome C leakage from mitochondria or plasma membrane standing death receptors, which culminates in the packaging of cellular content into apoptotic vesicles (Shalini et al., 2015). Cleavage of further protein substrates by caspase-1 impacts on innate defense by affecting cellular metabolism, phagosome maturation and autophagic clearance of intracellular pathogens (Rathinam & Fitzgerald, 2016). The latter is typically activated by autophagy adapters such as the ubiquitin reader Sequestosome-1 or the PRR NOD2, which trigger MAP1A/MAP1B Light Chain 3 (LC3) protein dependent intracellular membrane-engulfment of bacteria and autophagolysosome dependent neutralization (Deretic et al., 2013) (Figure 2). Autophagy activation can in turn dampen inflammasome formation and type I IFN immunity (Deretic et al., 2013), illustrating the complexity of the decision making networks in cell-autonomous antibacterial innate immunity.

The cellular innate immune response to bacterial pathogens is further shaped by cytokines produced by cells of the adaptive immune system, such as T- and NK-cells. The release of IL12 from PRR-activated macrophages for instance stimulates Th1-polarized CD4+ cells and CD8+ T-cells for type II interferon IFNγ production, which in turn stimulates defense against intracellular bacteria in macrophages and other cell types (Figure 2). IFNγ binding to the IFNγ receptor (IFNGR) activates a JAK/STAT signaling cascade, similar to the above described type I IFN signaling cascade (Schroder et al., 2004). IFNγ induced antibacterial adaptations, for example, include the production of reactive oxygen species or increased presentation of digested bacterial antigens to cytotoxic T cells (Schroder et al., 2004). Furthermore, Th2-polarized CD4+ lymphocytes upon TCR-based antigen sensing produce IL4 and IL13, which stimulate macrophage polarization towards an anti-inflammatory and repair-associated cell type (Atri et al., 2018). Thus, antibacterial immunity relies on an intricate interplay of innate and adaptive immune reactions, promoting bacterial clearance and re-establishment of homeostasis. Imbalances in the innate and adaptive immune pathways can result in attenuated immunity or over-shooting inflammatory reactions to bacterial pathogens and organ failure (Biswas & Lopez-Collazo, 2009; D’Elia et al., 2013). Therefore, numerous control mechanisms exist, which promote or negatively regulate the immune response (Biswas & Lopez-Collazo, 2009; D’Elia et al., 2013). Besides protein regulators, noncoding RNAs, including microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), are gaining increasing attention in this context. MiR-146 and miR-155 for instance constitute negative feed-back regulators, limiting TLR4-based immune-activation of mammalian macrophages (Janga et al., 2018; Schulte et al., 2013). Other miRNAs regulate autophagic bacterial clearance or are
highjacked by bacterial pathogens to their own advantage (Aznaourova & Schulte, 2021). While the roles of miRNAs in bacterial and viral infections have been studied in detail (K. Pawar, Sharbati, et al., 2016; Schulte et al., 2011; Sen Zhang et al., 2018; Zur Bruegge et al., 2016), research on lncRNAs in antibacterial host-defense is still at its infancy. Long noncoding RNAs are defined as transcripts with ≥200 nucleotides (nt) that lack protein-coding potential. Advances in high-throughput sequencing technologies in the 2000s have led to the discovery of thousands of lncRNAs in human cells and rodent models, only a fraction of which has been functionally characterized to date (M. Sun & Kraus, 2015). The approximately 20,000 lncRNAs encoded in the human genome constitute a heterogeneous group of enhancer-associated RNAs (eRNAs), intergenic transcripts, sense or antisense transcripts, acting in cis and in trans (Derrien et al., 2012; Kopp & Mendell, 2018). Mechanistically, lncRNAs can act as protein decoys, guides or scaffolds, for example, controlling chromatin factor positioning and thus transcription and genome compaction (Rinn & Chang, 2012). LncRNA PACER for instance decoys the p50 subunit of NFκB and thereby prevents formation of transcriptionally repressive NFκB p50 dimers (Krawczyk & Emerson, 2014). HOTAIR guides the Polycomb Repressive Complex 2 (PRC2) to target promoters to regulate HOX-gene dependent developmental programs (Rinn et al., 2007; Zhao et al., 2008). By bringing PRC2 and the Lysine Demethylase 1A (LSD1)/REST Co-repressor (CoREST) complex into special proximity, HOTAIR also serves as a scaffold RNA (Tsai et al., 2010). Besides their important roles in the nucleus, lncRNAs are also increasingly reported to participate in cytoplasmic processes, including translation, protein-ubiquitination or RNA turnover (Aillaud & Schulte, 2020). Thus, unlike miRNAs, lncRNAs do not act through a
common biochemical pathway but are mechanistically heterogeneous. Several lncRNAs have recently been described as regulators of sterile inflammatory responses to bacterial PAMPs and cellular defense against live bacterial pathogens.

2 | LncRNAs in Sterile Anti-Bacterial Immunity

Both professional immune cells, such as phagocytes, and tissue-forming cells, such as endothelial and epithelial cells, can initiate NFκB-dependent inflammatory responses to bacterial immune agonists. The defense programs mounted by these cell types differ depending on whether sterile bacterial PAMPs or live bacteria are sensed (see Section 3). Sterile antibacterial immunity can, for example, occur in response to remnants of a cleared infection or injected adjuvants (Hajam et al., 2017). In basic research, sterile bacterial PAMPs or synthetic PRR agonists are used to dissect the participation of immune-regulators in specific immune-signaling pathways. Several recent studies suggest nuclear and cytoplasmic lncRNAs to participate in the positive and negative control mechanisms balancing NFκB-dependent inflammatory responses to bacterial PRR-agonists.

2.1 | Positive Control of NFκB Driven Immunity upon PRR-activation

Phagocytes, such as monocytes or macrophages, play an essential orchestrating role in the antibacterial innate immune response (Kaufmann & Dorhoi, 2016). In human phagocytes, the cytokine IL1β is among the most highly induced pro-inflammatory mediators upon MyD88-pathway activation by bacterial LPS or lipoproteins. Stimulation of human monocytes with *Escherichia coli* LPS also induces expression of the nuclear-retained lncRNAs IL-1β-eRNA and IL-1β-RBT46. Both lncRNAs are transcribed from the same gene locus as IL1β and positively regulate IL1β expression (Ilott et al., 2014). In human macrophages, LPS furthermore induces the expression of nuclear lncRNA PACER, which prevents the formation of repressive p50 homodimers and thus promotes NFκB p50/p65 heterodimer formation and immune-gene transcription (Krawczyk & Emerson, 2014). Similar to PACER, lncRNA CARLR upon human macrophage activation with *E. coli* LPS promotes the expression of immune genes, via direct association with NFκB (Castellanos-Rubio et al., 2017). Importantly, CARLR is conserved and its immune-regulatory function extends to murine phagocytes as well (Castellanos-Rubio et al., 2017). At the level of promoter histone modification, the lncRNAs ROCKi and IL7-AS promote MyD88-dependent gene expression upon human macrophage stimulation with LPS and bacterial lipoprotein mimic Pam3CSK4, respectively (X. Liu et al., 2019; Q. Zhang et al., 2019). Of note, IL7-AS belongs to the few conserved immune-regulatory lncRNAs found both in human and murine cells (Roux et al., 2017).

In murine phagocytes, several lncRNAs with little indication for sequence conservation in human cells (Roux et al., 2017), were found to contribute to PAMP-triggered immunity. In murine macrophages, lncRNA-Tnfaip3 is upregulated in response to LPS and promotes NFκB-dependent gene expression by establishing an activatory High Mobility Group Box 1 (HMGB1)/NFκB complex at immune gene loci (S. Ma et al., 2017). Using an NFκB-GFP reporter system, additional lncRNAs were found to regulate NFκB-dependent immune gene expression in murine macrophages stimulated with bacterial LPS or bacterial lipoprotein analogue Pam3CSK4 (Covarrubias et al., 2017). While partially cytoplasmic lncRNA lincRNA-Cox2 was found to promote degradation of the NFκB inhibitor IκBα in the cytosol, lncRNA AK170409 seemed to be promote NFκB-dependent immune responses in the nuclear compartment (Covarrubias et al., 2017). Besides the positive control of IκBα degradation in the cytoplasm, linc-Cox2 seems to adopt both positive and negative regulatory roles in nuclear immune-gene transcription through association with Heterogeneous Nuclear Ribonucleoprotein hnRNPA/B and A2/B1 complexes (Carpenter et al., 2013) and the SWItch/Sucrose Non-Fermentable (SWI/SNF) chromatin remodeling complex (Hu et al., 2016). Another lncRNA controlling immune gene expression in response to LPS in murine macrophages is AS-IL1α, which acts to recruit RNA polymerase II to the promoter of the neighboring IL1α gene (Chan et al., 2015a). Further lncRNAs, such as FIRRE (Lewandowski et al., 2019; Y. Lu et al., 2017) or lncRNA-CCL2 (Jia et al., 2018) are induced in response to bacterial LPS and seem to promote pro-inflammatory murine phagocyte activation; their precise roles in this context, however, demand further investigation.

Besides pro-inflammatory, PRR-inducible lncRNAs, several immunosuppressive lncRNAs were found to be downregulated upon bacterial PAMP-stimulation, thereby contributing to the progression of the immune response. GAPLINC, for instance, is a functionally and positionally conserved lncRNA which is downregulated upon human and murine macrophage stimulation with various PRR ligands, including bacterial LPS (Vollmers et al., 2021). GAPLINC
In murine macrophages, exposure to bacterial LPS triggers the induction of several lncRNAs, acting to restrain NFκB deficiency enhanced baseline expression of NFκB dependent genes in human cells and mice. This pre-activation of immune-response genes protected GAPLINC-deficient mice from LPS-induced endotoxin shock—a fulminant inflammatory response syndrome, associated with high mortality (Vollmers et al., 2021). Other examples of lncRNAs suppressing premature immune responses, which are downregulated upon bacterial PAMP-stimulation, are lincRNA-EPS (Atianand et al., 2016) and Inc13 in murine phagocytes. While lincRNA-EPS interacts with hnRNPL, Inc13 associates with hnRNPD and Histone Deacetylase 1 (HDAC1). In both cases, lincRNA-protein complex formation counteracts immune gene activation by promoting repressive chromatin states at immune gene promoters (Castellanos-Rubio et al., 2016). In summary, several lncRNAs act in the nucleus and cytoplasm to foster MyD88-NFκB dependent immune gene expression upon activation of human and murine phagocytes by bacterial PRR ligands.

Of note, NFκB-dependent pro-inflammatory immune responses do not only rely on professional immune cells. Other cell types, such as vascular endothelial cells or mucosal epithelial cells vitally contribute to first-line inflammatory responses to bacterial pathogens (Lentsch & Ward, 2000; Luissint et al., 2016). Therefore, it is not surprising that several lncRNAs involved in antibacterial responses of immune cells are also engaged in epithelial immunity. Examples are PACER (Krawczyk & Emerson, 2014), CARLR (Castellanos-Rubio et al., 2017), IL7-AS (X. Liu et al., 2019; Roux et al., 2017), and FIRRE (Y. Lu et al., 2017), which positively regulate both phagocyte and epithelial NFκB-driven sterile immune responses to bacterial PAMPs. Other lncRNAs promote antiviral type I IFN defense pathways in epithelial and endothelial cells (Walther & Schulte, 2020), as well as immune-responses to live bacterial pathogens (see Section 3). Thus, a dense network of lncRNAs grants the induction of leukocyte, epithelial and endothelial host defense in response to bacterial PRR agonists. These cellular reactions need to be restricted to prevent from excessive immune-mediator production and inflammation-associated tissue damage and organ failure (Biswas & Lopez-Collazo, 2009; D’Elia et al., 2013). Several lncRNAs have been implicated in this process, as described in the following section.

2.2 Negative regulation of NFκB driven immunity upon PRR activation

In murine macrophages, exposure to bacterial LPS triggers the induction of several lncRNAs, acting to restrain NFκB dependent inflammatory responses. Mirt2, for instance, is induced upon TLR4-activation and negatively regulates the TLR-MyD88 pathway by impeding auto-ubiquitination and oligomerization of the signal transduction component TRAF6 (Du et al., 2017). In the nucleus, LPS-induced lncRNA Anti-IL1β inhibits IL1β expression by decreasing histone 3, lysine 4 (H3K4) tri-methylation at the promoter (J. Lu et al., 2013). LncRNA SeT is induced in response to LPS in murine macrophages to suppress the biallelic expression of tumor necrosis factor (TNFα) (Stratigi et al., 2015). In SeT deficient mice, increased TNFα production coincides with increased mortality upon LPS-injection-induced sterile endotoxemia (Stathopoulou et al., 2017). Besides limiting the acute induction of NFκB-dependent immune responses, lncRNAs also assist in the resolution of the inflammatory response to bacterial PAMPs, as evidenced by lncRNA IncFAO. This lncRNA is induced during the late LPS-response of murine macrophages and contributes to inflammation resolution by promoting mitochondrial hydroxacyl-CoA dehydrogenase beta (HADHB)-dependent fatty acid oxidation, which down-modulates macrophage inflammatory activity (Nakayama et al., 2020).

In a human macrophage model, lncRNA THRIL was reported to be downregulated upon stimulation with bacterial lipoprotein mimic Pam3CSK4. In complex with hnRNPL, THRIL was suggested to promote expression of pro-inflammatory mediator TNFα at the promoter level (Z. Li et al., 2014). Downregulation of THRIL upon macrophage-activation would thus inhibit TNFα production. In an independent RNA-seq study, however, expression of this lncRNA in human phagocytes could not be confirmed (Roux et al., 2017).

Like in phagocytes, other cell types such as epithelial and endothelial cells or fibroblasts employ lncRNAs to dampen NFκB-dependent immune responses. Sensing of bacterial LPS by human intestinal epithelial cells, for instance, leads to the induction of lncRNA TMC3-AS, which negatively regulates NFκB p65 binding to target gene promoters (Ye et al., 2020). In human endothelial cells, Inc-IL7R is induced upon sensing of LPS. Lnc-IL7R limits the inflammatory response by supporting the tri-methylation of H3K27 at immune gene promoters (Cui et al., 2014). In murine fibroblasts, Lethe is induced upon stimulation with TNFα, an inflammation mediator that is, for example, produced by phagocytes upon stimulation with bacterial or viral PAMPs. Lethe interacts with NFκB p65 and inhibits its binding to DNA, thereby restraining NFκB-dependent inflammatory responses (Rapicavoli et al., 2013).

Taken together, lncRNAs vitally participate in the positive and negative cellular control mechanisms preventing from attenuated and overshooting NFκB-dependent immune responses to bacterial PAMPs. Of note, in addition to NFκB-dependent immunity, lncRNAs also participate in the control of type I interferon immunity. The known roles of
In LncRNAs in this context, however, largely relate to viral infections (Walther & Schulte, 2020). An exception is the nuclear LncRNA LUCAT1, which was recently shown to be rapidly induced following LPS-stimulation of human monocytes, dendritic cells and macrophages, in an NFκB and JAK/STAT dependent manner (Agarwal & Vierbuchen, 2020). Loss- and gain-of-function experiments revealed LUCAT1 to restrain type I interferon stimulated gene expression. This seems to involve LUCAT1 recruitment to chromatin and an inhibitory interaction with STAT1 (Agarwal & Vierbuchen, 2020). The contribution of LUCAT1 to the clearance of life bacterial infections, however, remains to be determined. The already known functions of LncRNAs in the defense against living bacterial pathogens through interferon-activation and other pathways restraining bacterial colonization are described in the following sections.

3 | LncRNAs in antimicrobial IFNγ circuitries

In TH1 cells, CD8+ T-cells and NK cells, IFNγ production is promoted by the LncRNA IFNG-AS1, which is also known as NeST or as Tmevpg1 (Collier et al., 2012; Padua et al., 2016; Petermann et al., 2019; Stein et al., 2019; Vigneau et al., 2003). In murine cells, IFNG-AS1 was shown to promote activating histone methylation events at the IFNγ locus through a direct interaction with the methyltransferase WD Repeat Domain 5 (WDR5) (Gomez et al., 2013). Consequently, mice lacking IFNG-AS1 are more likely to succumb to Salmonella enterica Typhimurium infections than wild-type animals due to reduced IFNγ expression and increased bacterial burden (Gomez et al., 2013). In CD8+ T-cells it was shown that the expression of IFNγ and of the inflammation mediator TNFα is regulated by the T-cell—inhibitory molecule CD244 upon Mycobacterium tuberculosis infection (Y. Wang et al., 2015). CD244 induces the expression of a LncRNA, IncRNA-CD244, which directly interacts with the PRC2 subunit Enhancer of Zeste Homolog 2 (EZH2) to suppress IFNγ and TNFα expression at the chromatin-level (Y. Wang et al., 2015).

In phagocytes, stimulation with IFNγ activates the IFNGR-JAK–STAT signaling pathway, which was found to be regulated by LncRNA Sros1 in the context of L. monocytogenes infection (Xu et al., 2019). Mechanistically, L. monocytogenes upregulates miR-1, which targets cytoplasmic Sros1 for degradation. This in turn was suggested to release Sros1-dependent suppression of the RNA binding protein Cell Cycle Associated Protein 1 (CAPRIN1), which stabilizes Stat1 mRNA and increases STAT1 protein levels and thus IFNγ signaling. Thereby, inducible degradation of Sros1 contributes to clearance of intracellular bacteria (Xu et al., 2019). IFNγ also contributes to the polarization of macrophages towards a pro-inflammatory M1-like phenotype, as opposed to a rather anti-inflammatory phenotype induced by IL4. Knockdown of linc-Cox2 in murine RAW264.7 macrophages was shown to suppress the clearance of intracelluar Mycobacterium bovis BCG (Bacillus Calmette–Guérin) by inhibiting M1 polarization and nitric oxide production (W. Chen et al., 2019). Vice versa, linc-Cox2 overexpression enhanced binding of NFκB to the inducible Nitric Oxide Synthase (iNOS) promoter, thereby propelling nitric oxide production (W. Chen et al., 2019). Beyond linc-Cox2, in Mycobacterium tuberculosis infected murine macrophages, IFNγ pre-stimulation was found to pronouncedly alter the lncRNA expression profile (Roy et al., 2018). The relevance of this observation in the context of the course of bacterial infection, however, remains to be determined.
3.2 LncRNAs in autophagic clearance of bacteria

Autophagy is a central process in the maintenance of cellular homeostasis, promoting lysosomal breakdown of proteins or damaged organelles (Islam Khan et al., 2018). Autophagy also critically contributes to defense against intracellular bacteria, such as M. tuberculosis or the vaccine strain M. bovis BCG (Gutierrez et al., 2004) and is enhanced upon IFNγ stimulation (Ivashkiv, 2018). Recently, lncRNAs were found to play critical roles in control of autophagy under diverse pathological conditions (Bao et al., 2017; Z. Liu et al., 2016; Yang, Zhang, Li, & Liu, 2016c), including bacterial infections (Figure 2).

Maternally expressed lncRNA gene 3 (MEG3), for example, was shown to be significantly downregulated in IFNγ-treated THP1-derived macrophages challenged with M. bovis BCG. Knockdown of MEG3 fostered the induction of autophagy and promoted clearance of M. bovis (Pawar, Hanisch, Palma Vera, Einspanier, & Sharbati, 2016a). Interestingly, different from M. bovis infection, MEG3 is upregulated in human THP1-derived macrophages upon challenge with M. smegmatis, which is considered nonpathogenic, but not with M. avium, which is a facultative pathogen (Sharbati et al., 2019). Thus, MEG3 might play different roles in response to different mycobacterial species. PCED1B-AS is another lncRNA involved in the regulation of autophagy during mycobacterial infection. In CD14+ monocytes from patients with active tuberculosis PCED1B-AS levels were reduced, accompanied by decreased apoptosis and enhanced autophagy (M. Li et al., 2019). Mechanistically, PCED1B-AS was suggested to bind miRNA-155 and impair its function (M. Li et al., 2019). The downregulation of miR-155 targets Forkhead Box O3 (FOXO3) and Ras Homolog, MTORC1 Binding (RHEB) due to PCED1B-AS downregulation was suggested to increase LC3 expression and reduce caspase-3 levels, potentially explaining the observed effects on autophagy and apoptosis, respectively (M. Li et al., 2019). In response to microbial PAMPs, macrophages downregulate the long intergenic RNA-erythroid pro-survival (lincRNA-EPS), which functions as a repressor of immune gene expression (Atianand et al., 2016). Similar to PCED1B-AS1, lincRNA-EPS expression was found to be decreased in monocytes from patients with active pulmonary tuberculosis (PTB) (Ke et al., 2020). Knockdown of lincRNA-EPS inhibited apoptosis and enhanced autophagy in M. bovis BCG-infected murine RAW264.7 macrophages by promoting JNK/MAPK signaling (Ke et al., 2020). Taken together, the clearance of intracellular bacteria, such as M. tuberculosis, M. bovis or L. monocytogenes through the process of autophagy is tightly controlled by lncRNAs, directly impacting on autophagic activity of infected host cells or on the responsiveness to and production of autophagy inducer IFNγ.

3.3 LncRNAs in inflammatory responses to living bacteria

As already noted above, the type II interferon IFNγ not only promotes autophagy but also potentiates cellular immune responses to PRR ligands. The resulting production of inflammation mediators, such as IL1β, Cox2, IL6, or TNFα critically contributes to bacterial clearance but also to inflammatory pathologies if not properly controlled (Van Amersfoort et al., 2003). To avoid from attenuated or overshooting inflammation during antimicrobial immune responses, intricate regulatory RNA mechanisms have evolved, controlling the PRR-NFκB signaling pathways (Walther & Schulte, 2020). The nuclear lncRNA AS-IL1α, for instance, is upregulated upon L. monocytogenes infection in an NFκB dependent manner (Chan et al., 2015b). AS-IL1α in turn promotes the expression of IL1α by recruiting RNA polymerase II to the IL1α promotor (Chan et al., 2015b). Another lncRNA upregulated in murine macrophages upon L. monocytogenes infection is linc-Cox2 (Carpenter et al., 2013), which positively and negatively regulates distinct sets of immune genes through nuclear and cytoplasmic mechanisms (see Section 2.1). In human peripheral blood mononuclear cells (PBMCs) from patients with tuberculosis and in THP1-derived macrophages infected with M. tuberculosis Nuclear Paraspeckle Assembly Transcript 1 (NEAT1) was found to be upregulated compared to control group cells (Huang et al., 2018). Knockdown of NEAT1 in THP1-derived macrophages led to reduced IL6 but not TNFα levels after infection with M. tuberculosis and increased bacterial load (Huang et al., 2018). While these observations remain to be explained mechanistically, other studies using sterile PRR ligands found NEAT1 to displace the paraspeckle protein and transcriptional regulator SFPQ (Splicing Factor Proline And Glutamine Rich) from target immune gene promoters (Imamura et al., 2014; H. Ma, Ming, et al., 2017). An lncRNA known to be downregulated upon PRR-stimulation in phagocytes is lincRNA-EPS, which relieves hnRNPL-dependent suppression of immune gene expression at the promoter level (see Section 2.1). In line with these observations, in L. monocytogenes challenged macrophages and dendritic cells from lincRNA-EPS−/− mice increased levels of pro-inflammatory cytokines, such as TNFα and IL6 were detected (Agliano et al., 2019). In addition, increased expression
of iNOS and increased nitric oxide production was observed. Consequently, bacterial colony forming units (CFUs), recovered from liver and spleen of \textit{L. monocytogenes} infected lincRNA-EPS\textsubscript{−/−} mice, were reduced compared to wild-type animals (Agliano et al., 2019). Thus, PRR-dependent downregulation of lincRNA-EPS upon phagocyte infection serves to promote pro-inflammatory immune responses and bacterial clearance. In human blood-derived macrophages infected with the gram-negative bacterial pathogen \textit{Legionella pneumophila}, upregulation of the IncRNAs Macrophage Interferon-regulatory IncRNA 1 (MaIL1) and Linc01215 was observed (Aznaurova et al., 2020). MaIL1 is induced in a TLR4-NF\textsubscript{κB} dependent manner, but regulates TLR4-TRIF dependent type I interferon production. Thus, different from the IncRNAs depicted above, MaIL1 bridges the TLR-NF\textsubscript{κB} and the TLR-TRIF pathway. Mechanistically, MaIL1 associates with the ubiquitin reader and signaling adapter OPTN to promote OPTN and TBK1-kinase dependent phosphorylation of the IRF3 transcription factor and thus type I IFN expression. MaIL1 silencing in macrophages increased intracellular \textit{L. pneumophila} CFU counts, which could be reversed by the addition of recombinant type I IFN (Aznaurova et al., 2020). Taken together, several IncRNAs regulate the production of inflammation mediators and type I interferons in bacterially infected human and murine phagocytes in an NF\textsubscript{κB} dependent manner (Figure 3), thereby contributing to productive antibacterial defense.

In addition to phagocyte immunity, IncRNAs also regulate inflammatory reactions of epithelial cells and heterogeneous mucosal cell networks to living bacterial pathogens. \textit{Pseudomonas aeruginosa}, for instance is an opportunistic gram-negative pathogen, which can cause severe lung infections, especially in immune-compromised individuals (Sadikot, Blackwell, Christman, & Prince, 2005). RNA-seq analysis of \textit{P. aeruginosa} infected bronchial epithelial cells from cystic fibrosis (CF) patients compared to non-CF epithelial cells revealed 108 IncRNAs that were differentially expressed over the course of the infection. Two IncRNAs, MEG9 and BLACAT1, were validated to be significantly downregulated in CF cells after infection (Balloy et al., 2017). In lung tissue of \textit{P. aeruginosa} infected mice, another member of the maternally expressed gene family, the lncRNA MEG3, was found to be significantly downregulated in a TLR4-NF\textsubscript{κB} signaling dependent manner (R. Li & Fang, 2018). Downregulation of MEG3 transcript isofom 4 reduced IL1\textsubscript{β} expression, whereas MEG3-4 overexpression was found to promote excessive inflammation and propel bacterial infection.
colonization of murine lungs in vivo. Mechanistically, lncRNA MEG3-4 was suggested to suppress IL1β by competing with IL1β mRNA for miR-138 binding (R. Li & Fang, 2018). Many additional lncRNAs were shown to be regulated at the epithelial and endothelial barriers. Using a 3D reconstructed model of the human intestinal barrier, dozens of uncharacterized lncRNAs were shown to be regulated upon *Salmonella enterica* Typhimurium infection in the communicating intestinal epithelial cells, endothelial cells, monocytes and NK cells (Schulte, 2020). Similarly, in human brain microvascular endothelial cells (hBMECs) dozens of lncRNAs were up- or downregulated following infection with meningitic *E. coli* (R. Yang et al., 2016a). The roles of these lncRNAs in bacterial infection and host defense, however, remain to be determined. Taken together, phagocyte, epithelial and endothelial lncRNAs seem to vitally participate in the control of pro-inflammatory immune responses to live bacterial pathogens, with relevance to inflammation- and infection-induced pathologies.

### 3.4 LncRNAs linking bacterial infection to cancer

In the context of colorectal cancer (CRC), *Fusobacterium nucleatum* was identified as a gut microbe particularly enriched in the local tumor tissue environment (C. H. Sun et al., 2019). RNA-seq analysis of CRC cells exposed to *F. nucleatum* revealed 43 lncRNAs, which were upregulated in response to the bacterium, in addition to classic immune response genes such as CCL20 or IL8. Among these lncRNAs was Keratin 7 antisense RNA (KRT7-AS), which was induced in an NFκB dependent manner (S. Chen et al., 2020). Loss and gain of function assays revealed KRT7-AS to promote metastasis formation by CRC cells in nude mice by upregulating KRT7 (S. Chen et al., 2020). Thus, KRT7-AS is an example of an lncRNA acting at the interface of NFκB-triggered inflammation and cancer in the context of bacterial infection. Besides colorectal cancer, *F. nucleatum* is also believed to contribute to oral squamous cell carcinoma (OSCC). Upon stimulation of OSCC cells with *F. nucleatum*, the lncRNA MIR4435-2HG-5p was found to be upregulated and to interact with miR-296-5p. Inactivation of this miRNA by MIR4435-2HG-5p was suggested to activate of serine/threonine kinase Akt2, which contributes to epithelial–mesenchymal transition—a process critically contributing to tumorigenesis (Shuwei Zhang et al., 2020). Another bacterium, which is often associated with cancer is the gastric pathogen *Helicobacter pylori*. Recently, lncRNAs were suggested to participate in the development of *H. pylori* induced gastric cancer. The lncRNA Inc-GNAT1, for instance, is downregulated upon *H. pylori* infection in a dose dependent manner in normal gastric and gastric cancer cells. Lnc-GNAT1 overexpression inhibits the migration of gastric cancer cells (L. Liu, Shuai, Li, Zhu, & Li, 2018). In line with this observation, IncRNA-GNAT1 was found to negatively regulate the Wnt/β-Catenin pathway, which promotes cancer cell proliferation (L. Liu et al., 2018; Mao et al., 2014). In vivo experiments in which Inc-GNAT1-1 overexpressing cells were subcutaneously injected into nude mice showed reduced tumor growth (L. Liu et al., 2018). Besides Inc-GNAT1-1, the lncRNA SNHG17 was shown to be upregulated in GES-1 cells upon *H. pylori* infection. Furthermore, elevated SNHG17 expression was detected in *H. pylori*–positive gastric cancer tissue (Han et al., 2020). *H. pylori*-induced SNHG17 expression was suggested to contribute to tumorigenesis by deregulating Non-POU Domain Containing Octamer (NONO) and miR-3909/Ring Finger Protein 1/Rad51 dependent pathways impacting on the DNA repair machinery (Han et al., 2020).

In summary, the lncRNAs KRT7-AS, MIR4435-2HG, IncRNA-GNAT1 and SNHG17 contribute to bacterial colonization induced tumorigenesis in the context of colorectal and gastric cancer by regulating cell proliferation, invasion and DNA repair pathways (Figure 4).

### 3.5 LncRNAs in other processes linked to bacterial infection

Further lncRNAs have been implicated in host interplay with bacterial pathogens, which cannot be assigned to the classic antimicrobial defense pathways or infection-induced pathologies portrayed above. In HeLa cells infected with *Salmonella* Typhimurium, for instance, the unstable lncRNA NEAT1 isoform Neat1v2 was shown to be upregulated due to diminished Nuclear Exosome Targeting (NEXT) complex dependent nuclear RNA decay (Imamura et al., 2018). Challenge with *Salmonella* mutants lacking the pathogenicity islands SPI-1 and SPI-2, required for invasion and intracellular replication, as well as with heat-killed *Salmonella*, LPS or flagellin did not affect Neat1v2 expression (Imamura et al., 2018). This suggests, that Neat1v2 upregulation depends on intracellular bacterial replication or bacterial manipulation. Cells deficient in NEAT1v2 displayed reduced expression of immune genes, such as TNFSF9, CCL2 or
CSF1 as well as increased Salmonella load, indicating that the upregulation of NEAT1v2 serves to promote antibacterial defense, rather than a bacterial infection strategy (Imamura et al., 2018). Other lncRNAs were found to be induced in Rickettsia infected cells. Bacteria of the genus Rickettsia, transmitted by ticks, cause spotted fever, a disease characterized by systemic symptoms, such as fever and muscle aches (Wood & Artsob, 2012). In lung tissue from mice infected with the model species R. conorii, RNA-seq analysis identified the two enhancer lncRNAs NONMMUT013718 and NONMMUT024103, which promote the expression of Inhibitor Of DNA Binding 2 (Id2) and Apolipoprotein Apol10b, likely by interacting with the promoters of these genes (Chowdhury et al., 2019). Whereas Id2 belongs to a family of transcription factors involved in cellular differentiation (Sikder, Devlin, Dunlap, Ryu, & Alani, 2003), the Apolipoproteins L family, to which Apol10b belongs, has been implicated in TLR3-TRIF- and parasite-induced cell death (Smith & Malik, 2009; Uzureau et al., 2016). The relevance of these regulations in the context of Rickettsia infections, however, remains to be determined.

In addition to their direct implications in bacteria-host interplay, lncRNAs were suggested as clinical markers in the context of bacterial infections. For instance, expression of the lncRNAs MIR3945HG V1 and MIR3945HG V2, which are upregulated in primary human macrophages infected with M. tuberculosis, was found to be elevated in PBMCs from tuberculosis compared to control group patients (X. Yang et al., 2016b). Similarly, the expression of lncRNA MaIL1, which promotes type I IFN expression in phagocytes, was found to be elevated and linearly correlated with IFN levels in bronchoalveolar lavage samples of pneumonia patients (Aznaurova et al., 2020). In a genome wide association study (GWAS) a polymorphism in lincRNA gene AC011288.2 could be associated with bacteremia caused by Streptococcus pneumoniae in Kenyan children. The AC011288.2 lncRNA was found to be primarily expressed in neutrophils, in line with its anticipated role in antibacterial defense (Kenyan Bacteraemia Study Group et al., 2016). The cellular function of this lncRNA, however, remains to be determined. Polymorphisms in other lncRNAs have been associated with chronic and acute inflammatory diseases (Aznaurova, Schmerer, Schmeck, & Schulte, 2020). This adds additional evidence for an important role of lncRNAs in host defense and misguided immune responses in the context of bacterial infections (Table 1).
| Pathway | IncRNA | Pathogen | Cell type | Regulation | Function | Mechanism | Organism | References |
|---------|--------|----------|-----------|------------|----------|-----------|----------|------------|
| IFNγ circuitries | IFNG-AS1 | *Salmonella* | Mice | Up | IFN-γ regulation | Promotion of histone methylation at the IFNγ locus through interaction with WDR5 | hsa; mmu | Gomez et al. (2013) |
| | IncRNA-CD244 | *Mtb* | CD8⁺ T-cells | Up | Regulation of TNF-α and IFN-γ expression | Interaction with PCR2 → repressive chromatin state | hsa | Wang et al. (2015) |
| | IncRNA-SROS1 | *L. monocytogenes* | Macrophage | Down | Post-transcriptionally regulation | Blocks the binding of Stat1 mRNA to the RBP CAPRIN1, which stabilizes Stat1 mRNA | mmu | Xu et al. (2019) |
| | IncRNA-Cox2 | *M. bovis BCG* | Macrophage | Up | Clearance and regulation of intracellular BCG | Regulation of M1 polarization/nitric oxide production; mediation of NF-κB p65 and p50 to the iNOS promoter | mmu | Chen et al. (2019) |
| Autophagy | MEG3 | *M. bovis BCG* | Macrophage | Down | Autophagy | Unknown | hsa | Pawar et al. (2016a) |
| | MEG3 | *M. smegmatis* | Macrophage | Up | Suggested to decrease TGF-β levels | Unknown | hsa | Sharbati et al. (2019) |
| | PCED1B-AS | Active Tuberculosis | Macrophage | Down | Lessens apoptosis and enhances autophagy in monocytes | Endogenous sponge to block miR-155 expression in macrophages by directly binding to miR-155 | hsa | Li et al. (2019) |
| | lincRNA-EPS | Pulmonary tuberculosis; *M. bovis BCG* | Monocyte; macrophage | Down | Regulation of apoptosis and autophagy | JNK/MAPK signaling pathway | mmu | Ke et al. (2020) |
| NFκB | IncRNA AS-IL1α | *L. monocytogenes* | Macrophage | Up | IL-1α regulation | RNA polymerase II recruitment to the IL-1α promoter | mmu | Chan et al. (2015a, 2015b) |
| | Linc-Cox2 | *L. monocytogenes* | Macrophage | Up | Regulation of immune genes | Direct interaction with hnRNP-A/B and hnRNP-A2/B1 | mmu | Carpenter et al. (2013) |
| | NEAT1_1; NEAT1_2 | *Mtb* | PBMCs; macrophage | Up | Regulates IL-6 expression | Unknown | hsa | Huang et al. (2018) |
| | lincRNA-EPS | *L. monocytogenes* | Macrophage | Down | Regulation of IRGs | Direct interaction with hnRNPL | mmu | Atianand et al. (2016) |
| | | | Macrophage; dendritic cell | Down | Regulation of TNF-α and IL-6; suppression of bacterial clearance and host defense responses | | mmu | Agliano et al. (2019) |
| Pathway | lncRNA | Pathogen | Cell type | Regulation | Function | Mechanism | Organism | References |
|---------|--------|----------|-----------|------------|----------|-----------|----------|------------|
| MaIL1   | L. pneumophila | Macrophage | Up | Regulation of type I interferon | Associates with ubiquitin and OPTN to promote phosphorylation of the IRF3 transcription factor | hsa | Aznaourova and Schulte (2021) |
| MEG9 BLACAT1 | P. aeruginosa | Bronchial primary epithelial CF cells | Down | Unknown | Unknown | hsa | Balloy et al. (2017) |
| MEG3-4 | P. aeruginosa | Macrophage; epithelial cells; lung | Down | miRNA decoy | Infection shuts down MEG3-4 to release miR-138 to repress IL-1β and contain inflammation | mmu | Li et al. (2018) |
| Cancer associated KRT7-AS | F. nucleatum | CRC cells | Up | Promotion of metastasis through upregulation of KRT7 | Unknown | mmu | Chen et al. (2020) |
| MIR4435-2HG-5p | F. nucleatum | OSCC | Up | inactivation of miR-296-5p and activation of Akt2 | Unknown | hsa | Zhang et al. (2020) |
| IncRNA GNAT1-1 | H. pylori | Gastric cancer cell | Down | Regulation of invasion and migration of gastric cancer cells | Regulation of Wnt/β-Catenin pathway protein expression | hsa | Liu et al. (2018) |
| SNHG17 | H. pylori |GES-1; gastric cancer tissue | Up | Directly interacting with miR-3909 and NONO | Altering DNA repair via SNHG17/ NONO and SNHG/miR-3909/ RING1/Rad51 pathways | hsa | Han et al. (2020) |
| Other processes NEAT1v2 | Salmonella | HeLa | Up | Regulation of immune genes; promotion of antibacterial defense | Diminished NEXT exosome-dependent nuclear RNA decay | hsa | Imamura et al. (2018) |
| NONMMUT013718 NONMMUT024103 | R. conorii | Macrophage; endothelial cell | Up | Promote the expression of Id2 and Apol10b | Interaction with promoter region | mmu | Chowdhury et al. (2019) |
| MIR3945HG V1, MIR3945HG V2 | Mtb | Macrophage | Up | Unknown | Unknown | hsa | Yang et al. (2016a, 2016b) |
| AC011288.2 | S. pneumoniae | Neutrophils | Elevated | Unknown | Polymorphism could be associated with bacteremia | hsa | The Kenyan Bacteraemia Study Group et al. (2016) |
Although only a fraction of the thousands of lncRNA genes annotated in human and rodent genomes has been characterized to date, important roles in host defense against bacterial pathogens are emerging. The roles of lncRNAs in this context range from positive and negative control of pro-inflammatory cytokine and interferon production to the regulation of autophagic clearance and nitric oxide mediated killing of intracellular bacteria (Table 1). In the context of cell-invasive bacterial infections, many lncRNAs seem to be engaged in the control of IFNγ and autophagy dependent antimicrobial defense. Although such lncRNAs have so far only been studied in connection with selected bacterial species, it can be assumed that their functions can be generalized to infections with other bacterial pathogens. For example, IFNγ production was shown to be regulated by lncRNAs such as IFNG-AS1 or lncRNA-CD244 during S. Typhimurium and mycobacterial infections, respectively (Gomez et al., 2013) (Y. Wang et al., 2015). Due to the central role of IFNγ in the activation of antibacterial reactions directed against a broad spectrum of intracellular bacteria, including reactive oxygen species production and enhanced autophagic clearance (Ivashkiv, 2018), these lncRNAs are likely to be involved in host defense against other intracellular pathogens such as L. monocytogenes or L. pneumophila as well. Besides type II IFN immunity, lncRNAs have also been implicated in host defense through type I IFNs. LncRNA MaIL1, for instance promotes type I IFN dependent defense against intracellular L. pneumophila (Aznaurova et al., 2020). Similar to type II IFN controlling lncRNAs, the antibacterial function of MaIL1 likely extends to other intracellular bacteria. Furthermore, the many lncRNAs implicated in type I IFN immunity in the context of viral infection (Vierbuchen & Fitzgerald, 2020) might be engaged in antimicrobial defense as well. At present, however, a systematic analysis of the contributions of known type I and II IFN regulatory lncRNAs to defense against bacterial pathogens is missing. The same holds true for lncRNAs involved in the NFκB-dependent pro-inflammatory response to bacterial agents.

Of note, both interferon and NFκB driven immune responses can become detrimental and contribute to life-threatening pathologies during bacterial infections, such as pneumonia and sepsis (S. F. Liu & Malik, 2006; Stifter & Feng, 2015). Further research is needed to reveal and therapeutically harness the roles of lncRNAs in this context. Although therapeutic targeting of lncRNAs for the treatment of human diseases has not yet been achieved, the FDA-approval of mRNA targeting drugs such as Eterplisien or Mipomersen (Shen & Corey, 2018) makes clinical lncRNA targeting seem feasible. Clinically approved RNA antagonists function as antisense inhibitors, composed of stable nucleic acid analogues. Often used analogues are for example Locked Nucleic Acid (LNA), Peptide Nucleic Acid (PNA) or Phosphorodiamidate Morpholino (PMO) (Shen & Corey, 2018). Recently, we showed that antisense PNA oligomers, coupled to octaarginine as a cell-penetrating peptide, can sterically block the NFκB upstream regulator miR-155 in human blood-derived macrophages at submicromolar concentration (Linden et al., 2021). This microRNA acts as a viral host factor and as a fine-tuner of cytokine production in antibacterial innate immunity (Linden et al., 2021; Shen & Corey, 2018; Skalsky & Cullen, 2010). Similar approaches might enable steric or splice-switching mediated inhibition of lncRNAs controlling cytokine and interferon production during bacterial infection. Besides RNA antisense inhibitors, advances in the development of small molecule RNA inhibitors have recently garnered much attention (Hargrove, 2020). To assess the potential of antisense or small molecule noncoding RNA antagonists as therapeutics in the context of bacterial infections, pre-clinical studies with small animal in vivo models are needed. The limited primary sequence conservation of lncRNAs between humans and rodents (Roux et al., 2017) could be a major obstacle in this context. Among the few immune-regulatory lncRNAs found to be conserved between humans and mice, so far, are CARLR, IL7-AS and GAPLINC, which regulate pro-inflammatory cytokine production (Castellanos-Rubio et al., 2017; Roux et al., 2017; Vollmers et al., 2021). An important task for the future will be to identify further locus- or structure conserved orthologues and functional equivalents of human immune-regulatory lncRNAs in mice. This will not only benefit a better understanding of their in vivo relevance during bacterial infection but also of their utility as therapeutic targets for antisense and small molecule RNA inhibitor approaches.

Besides their therapeutic relevance, another open question is whether lncRNAs are employed by bacterial pathogens to manipulate host immune pathways to their own advantage, as previously documented for viruses. HIV for instance induces expression of lncRNA SAF in infected macrophages to counteract caspase-3/7 dependent cell death (Boliar et al., 2019). Influenza A promotes expression of host lncRNA IPAN, which stabilizes viral RNA polymerase (J. Wang et al., 2019). Although lncRNA manipulation by microbial pathogens remains to be determined, bacteria are well known to manipulate other types of host noncoding RNA. S. Typhimurium, for instance, downregulates miR-15 family members to derepress cyclin D1 and promote G1/S transition, which is required for intracellular replication of the pathogen (Maudet et al., 2014). Thus, the subversion of host regulatory RNA circuits by viral and bacterial infectious agents is a known colonization strategy. Of note, research on lncRNAs in bacterial infection is still at its infancy,
which might explain, why so far, bacterial manipulation of lncRNA expression to the advantage of the pathogen has not been discovered. The application of specialized RNA-seq and cell sorting approaches enabling the dissection of the strategies of bacteria inside or at host cells they physically interact with (Schulte, Heinrich, Janga, Schmeck, & Vazquez, 2018; Westermann et al., 2016), could help to determine lncRNA-based bacterial virulence strategies.

Irrespective of bacterial manipulation, the many uncharacterized lncRNAs regulated in host cells challenged with bacterial immune agonists and live bacteria suggest that more functions of this class of regulatory RNA in host pathogen interplay will be revealed in the near future. The already known roles of lncRNAs suggest that the intricate RNA circuits promoting bacterial clearance and suppressing excessive immune responses are even more complex than previously thought. Further research on these regulatory RNA networks will certainly promote an improved, therapy-relevant understanding of the strategies of the immune system to cope with invading pathogens while preventing from immune-associated life-threatening conditions, such as sepsis and multi-organ-failure.

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CONFLICT OF INTEREST
The authors declare no competing interests.

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Data sharing is not applicable to this article as no new data were created or analyzed in this study.

AUTHOR CONTRIBUTIONS
Nils Schmerer: Conceptualization; visualization; writing-original draft; writing-review & editing. Leon Schulte: Conceptualization; supervision; visualization; writing-original draft; writing-review & editing.

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