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Crosstalk between microbiota, pathogens and the innate immune responses

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ABSTRACT

Research in the last decade has convincingly demonstrated that the microbiota is crucial in order to prime and orchestrate innate and adaptive immune responses of their host and influence barrier function as well as multiple developmental and metabolic parameters of the host. Reciprocally, host reactions and immune responses instruct the composition of the microbiota. This review summarizes recent evidence from experimental and human studies which supports these arms of mutual relationship and crosstalk between host and resident microbiota, with a focus on innate immune responses in the gut, the role of cell death pathways and antimicrobial peptides. We also provide some recent examples on how dysbiosis and pathogens can act in concert to promote intestinal infection, inflammatory pathologies and cancer. The future perspectives of these combined research efforts include the discovery of protective species within the microbiota and specific traits and factors of microbes that weaken or enforce host intestinal homeostasis.

1. Microbiota and innate immune recognition

Research in the last 10 years has convincingly demonstrated that the microbiota is crucial in order to prime and orchestrate innate and adaptive immune responses of their host and influence multiple developmental, metabolic and even neurological parameters of the host. This process starts shortly after birth (Lotz et al., 2006; Koenig et al., 2011; Fulde and Hornef, 2014) and is achieved by multiple, frequently redundant signals from transiently colonizing and residential microbial communities. In the host, this influences various parameters of health and disease and reverberates back on the microbiota. In some cases, the molecular nature of microbial signals and their mode of signaling have already been described and characterized at a biochemical level [see article Structure and Function: Lipid A modifications in commensals and pathogens by Frick and Autenrieth]. According to current knowledge, most of these signals address specifically and redundantly multiple innate immune receptors and initiate the respective downstream signaling pathways, which can be either activating or inhibitory (Chamy et al., 2015). The initial recognition of microbial presence occurs at the first line of defence, the epithelial cells, and in numerous other resident and migrating cell types that prime and modulate the immune response. The net outcome is then determined by the cellular integration of these multiple divergent or synergistic signals. Examples for microbial cues include Toll-like receptor (TLR) and lectin ligands (Kubinak and Round, 2012; El Chamy et al., 2015), and inflammasome activators such as microbial nucleotides and secretion system components (Sellin et al., 2015). Relevant models to dissect the host and bacterial factors and the contribution of single microbes of the microbiota or pathogens to the net outcome of specific colonization included germ-free animals. For comparative purposes, one frequently used setup are germ-free animals either left without intervention or mono-colonized using specific pathogens or components of the resident microbiota (Lichtman et al., 2015), or colonized with defined parts of the commensal microbiota derived from humans or mice (Galipeau et al., 2015; Collins et al., 2015; Hsiao et al., 2014). Recently, these approaches have also been instrumental in identifying specific microbial molecules or species that are beneficial in the face of pathogen attack or immune deregulation (Collins et al., 2015; Buffie et al., 2015; Underwood et al., 2014).

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It goes without saying that with a naturally changing microbiota over the lifetime of a host (Lakshminarayanan et al., 2014; Bischoff, 2016) the multitude of signals and their net outcome with respect to the immune response will also change. This is particularly important when considering the effect of antibiotic treatment, which can permanently change the microbiota, in particular in young children, either with regular exposure to subinhibitory levels of antibiotics or sometimes even with only one or few pulses of antibiotic regimens. In animal models, this hypothesis has been corroborated (Cho et al., 2012; Nobel et al., 2015).

2. Impact of the microbiota on host cell death pathways during intestinal inflammation and microbial infection

The abundance of trillions of beneficial commensal microorganisms in the gastrointestinal tract that reside together with cells of the gut-associated immune system requires epithelial surfaces as an effective barrier in order to define host–microbial interaction and to conserve tissue homeostasis. This physical and biochemical barrier is established by the tight contact of intestinal epithelial cells through tight junctions and the mucus layer. Highly specialized intestinal epithelial cells (IEC), such as Paneth and goblet cells provide innate immune functions by producing antimicrobial peptides (AMP) and highly glycosylated mucins that not only hamper access and survival of bacteria directly adjacent to the epithelium but can also instruct the microbial community within the gut (Specian and Oliver, 1991; Salzman, 2010). The microbiota, in its turn, shapes and strengthens the intestinal mucus barrier in the colon (Jakobsson et al., 2015). Furthermore, intestinal epithelial cells not only create a physical barrier, they can also sense information from microbial communities in the gut lumen and respond to microbial stimuli by coordinating immune responses, ranging from tolerance to anti-pathogen immunity (Rescigno, 2011). The association of microbial dysbiosis, increased bacterial translocation and intestinal inflammation, as seen in mouse models or human patients suffering from intestinal pathologies, suggests a central role for dysregulated intestinal barrier function in the pathogenesis of gastrointestinal inflammation and infection (Gunther et al., 2013). Barrier dysfunction and inflammation are strongly associated with a dysregulation of intestinal epithelial cell death (Gunther et al., 2011; Welz et al., 2011; Wittkopf et al., 2013; Takahashi et al., 2014; Gunther et al., 2015; Lopez-Posadas et al., 2016). While epithelial cell death has been demonstrated to promote intestinal inflammation, clearance of infected host cells by either apoptosis or other forms of programmed cell death is a pivotal step during infection to inhibit microbial replication and survival, since infected cells can be quickly expelled from the epithelium in order to prevent persistent infection. However, many bacterial pathogens are capable of colonizing and invading the epithelium, suggesting that they have developed strategies to antagonize the cell-death-mediated defense (Kim et al., 2010).

Until recently, apoptosis (Becker et al., 2013) and pyroptosis mediated by inflammasome activation (Jorgensen and Miao, 2015) were considered as the principal mechanisms of programmed host cell death. However, a recent addition to the concept of regulated cell death has been the discovery of necroptosis (Vandenabeele et al., 2010). Mouse and human studies have demonstrated that elevated levels of epithelial apoptosis contribute to intestinal inflammation. For example, mice deficient in the gene NEMO (nuclear factor kappa B (NF-κB) essential modulator) specifically in IECs (NEMO$^{\Delta IEC}$ mice) are characterized by the development of chronic colitis shortly after birth (Nenci et al., 2007), which appeared to be mediated by excessive apoptosis. NF-κB signaling, downstream of microbiota-driven innate immune recognition pathways, is known to be important for the activation of pro-survival genes (Kreuz et al., 2001; Lawrence, 2009), and its impairment in general results in an increased susceptibility to colitis (Wullaert et al., 2011). More recent studies have demonstrated that inhibition of caspase-8-dependent extrinsic apoptosis in IECs results in the activation of highly inflammatory necroptosis (Gunther et al., 2011; Welz et al., 2011). Opposed to apoptotic cells, cells undergoing necroptosis show morphological features of necrosis, resulting in cellular lysis accompanied by the release of danger-associated molecular patterns (DAMPs) that promote intestinal inflammation. This effect leading to severe colitis has been demonstrated in mice with an intestinal epithelial cell specific deletion of caspase-8 (Casp8$^{\Delta IEC}$ mice) or its adapter FAS-associated death domain protein (FADD$^{\Delta IEC}$ mice) (Gunther et al., 2011; Welz et al., 2011).

Necroptosis has also been described in macrophages during intestinal infection of mice with *Salmonella* Typhimurium (Robinson et al., 2012). Interestingly the extrinsic apoptosis pathway in these mice was not genetically manipulated, corroborating previous evidence that various, preferentially intracellular, pathogens may be able to inhibit extrinsic apoptosis.

Excessive epithelial cell death accompanying intestinal inflammation and infection has been described during infection with pathogenic bacteria such as *Salmonella* Typhimurium (Paesold et al., 2002) or *Citrobacter rodentium* (Wittkopf et al., 2015) and during viral, e.g. by rotaviruses (Pott et al., 2011), or parasite infection (*Cryptosporidium parvum*) (Foster et al., 2012). Cytokines (e.g. TNFα) provoked by pathogen infections, MAMPs (Microbe-Associated Molecular Patterns) or DAMPs, can act in concert with other cues to exacerbate cell death and intestinal inflammation (Gunther et al., 2013; Takahashi et al., 2014). For example *Salmonella* flagellin induces TNFα production that further promotes tissue injury (Arnold et al., 1993). In line with these mouse studies, epithelial cell death returned to control levels accompanied by increased barrier function when patients suffering from Crohn's disease underwent anti-TNF therapy, suggesting a pivotal role of TNFα in epithelial cell death (Zeissig et al., 2004). Besides TNFα, interferons (IFNs) play a critical role in the antimicrobial and antiviral host defense. Deregulated IFN production results in a cascade of cell death and inflammation that can lead to tissue injury and diseases in the context of pathogen defense (Malireddi and Kanneganti, 2013). Yet, the functional role of different cytokines in promoting either less-inflammatory epithelial apoptosis or inflammatory necroptosis remains to be elucidated. By contrast, several recent studies have extensively investigated the consequence of pattern recognition receptor (PRR) signaling on cell death of intestinal epithelial cells (Gunther et al., 2014). As one recent example, TLR3 ligation in intestinal epithelial cells induced either apoptosis or necroptosis (McAllister et al., 2013; Gunther et al., 2015). Mice quickly recovered from a transient increase of TLR3-triggered epithelial apoptosis, whereas TLR3-mediated necroptosis resulted in a complete breakdown of the intestinal barrier, leading to a lethal toxic spread of luminal bacteria into systemic sites (Gunther et al., 2015). Using primary intestinal epithelial organ cultures (organoids), the authors clarified that TLR3-triggered epithelial cell loss and barrier impairment does not require cytokine production by immune cells (Gunther et al., 2015).

The analysis of direct bacterial–epithelial interactions has been hampered by a lack of suitable intestinal epithelium culture systems. While *Salmonella* infection can promote both caspase-dependent and -independent programmed cell death in the intestine (Schauser et al., 2005), the direct mechanism of bacterially induced epithelial cell death still has to be elucidated. Recently, one study took advantage of the organoid culture system to study the pathophysiology of bacterial–epithelial interaction after *Salmonella* infection (Zhang et al., 2014). This study clearly demonstrated that *Salmonella* induces disruption of the epithelial barrier by