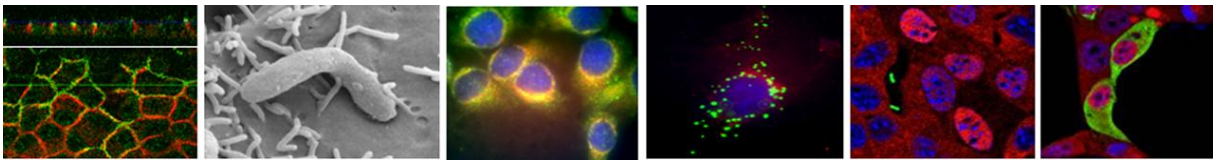
INNATE IMMUNE RECOGNITION AND ANTIMICROBIAL HOST RESPONSES BY INTESTINAL EPITHELIAL CELLS –



1. INNATE IMMUNE RECOGNITION BY INTESTINAL EPITHELIAL CELLS

The main research focus in our laboratory deals with the molecular basis, functional importance and regulation of intestinal epithelial innate immune recognition. It also includes the activation of host defence mechanisms in response to infection with enteropathogenic microorganisms as well as mechanisms that prevent inappropriate activation by the enteric microbiota. A particular emphasis is placed on the fetal-postnatal transition; i.e. the postnatal development of the enteric microbiota, the establishment of a stable host-microbial homeostasis after birth and the accompanying enhanced susceptibility to infection with certain enteric pathogens. In addition, constitutive and inducible mechanisms of epithelial antibacterial host defence such as antimicrobial peptides are a focus of our research (see below).

Starting point of our work was the analysis of a differentiated and polarized intestinal epithelial cell line that expressed the Toll-like receptor (TLR)4 and exhibited a surprising susceptibility to the ligand lipopolysaccharide (LPS) produced by gram-negative bacteria (Hornef et al., 2002). Further analyses revealed interesting differences in the cellular mechanisms of LPS recognition between myeloid and epithelial cells (Hornef et al., 2003). The functional importance of ligand internalization for epithelial activation was later confirmed (Duerr et al., 2009; Chassin et al., 2010).

We then turned to the *in vivo* analysis of enterocytes using new methods to isolate and analyse primary intestinal epithelial cells. We confirmed TLR4 expression and LPS susceptibility and made the striking observation of a transient acquired period of reduced LPS susceptibility during the postnatal period. Reduced ligand susceptibility was caused by a sustained posttranscriptional down-regulation of epithelial *interleukin 1 receptor associated kinase* (IRAK)-1, an essential signaling molecule of TLR-mediated cell stimulation (Lotz *et al.*, 2006). Both proteasomal/lysosomal degradation and microRNA mediated translational repression cause reduced epithelial IRAK1 protein levels after birth (Chassin *et al.*, 2010). In contrast, IRAK1 levels normalize after weaning and epithelial TLR stimulation significantly contributes to homeostatic epithelial signalling (Stockinger et al., 2014). Interestingly, IRAK1 protein levels rise in the adult epithelium following transient ischemia due to SUMOylation and a change in the ubiquitination (K48 to K63) pattern. This increase is functionally relevant and contributes to the postischemic tissue damage (Chassin et al., EMBO Mol Med, 2012). Thus the level of IRAK1 protein appears to critically determine the epithelial innate immune sensitivity.

Also the double stranded RNA receptor TLR3 is found at the intestinal epithelium. It is the only TLR that signals independent of the adaptor molecule MyD88 and IRAK1. Consistent with a reduced innate immune sensitivity in the neonate epithelium, TLR3 expression in the neonate is severely reduced. The functional relevance of low TLR3 expression in the neonate host is illustrated by the enhanced susceptibility to rotavirus infection in neonate mice but also adult mice deficient in TLR3 or the adaptor molecule TRIF (Pott et al., 2012). Virus infection of

epithelial cells induces local expression of interferons; among them both type I (α/β) and interferon-λ. Strikingly, interferon-λ appears to specifically protect the epithelium leading to significantly enhanced rotavirus replication in interferon-λ receptor (IL-28R) deficient animals. In contrast, type I interferon stimulates antiviral protection of lamina propria cells (Pott et al., 2011).

Epithelial cells also express Nod2, the receptor for muramyl di-peptide (MDP), a molecular motif in the peptidoglycan layer of the outer bacterial cell wall. Secretion of the amidase PGLYRP-2 by intraepithelial lymphocytes reduces the stimulatory potential of peptidoglycan released by the enteric microbiota (Duerr et al., Muc Immunol., 2011). Nod2 recognizes epithelial infection with *Listeria monocytogenes*, although only after lysis of the endosomal membrane and entry to the epithelial cytosol. Strikingly, although innate immune stimulation is restricted to the infected cells, mainly the surrounding non-infected cells respond to the infection with cytokine secretion. The mechanism of this epithelial cell-cell communication was shown to rely on NADPH oxidase 4 (NOX4) mediated reactive oxygen species (ROS) (Dolowschiak *et al.*, PLoS Pathogens, 2010) facilitating a coordinated epithelial response to focal infection.

Oral infection of neonate mice with the non-typhoid *Salmonella enterica* results in rapid colonization due to the absence of a diverse and competitive enteric microbiota. Surprisingly, we observed *Salmonella pathogenicity island* (SPI)1-dependent enterocyte invasion and the generation of intraepithelial microcolonies. Salmonella invasion induced TLR4 and 9 stimulation and caused a potent epithelial innate immune activation (Zhang et al., 2014).

Current work continues on the particular susceptibility of the neonate intestinal epithelium to infection and its long-term consequences.

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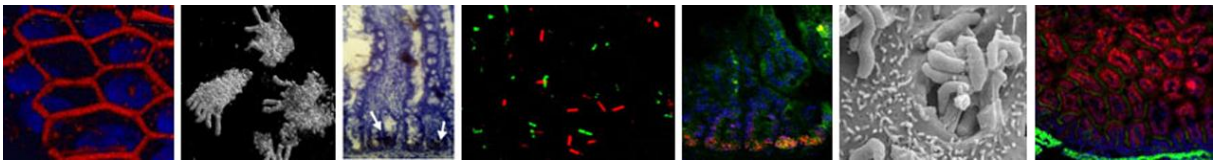

2. Epithelial Innate immune host defenses: Enteric antimicrobial peptides

The immediate host defence of all multicellular organisms including mammals relies to a great extent on the production and targeted secretion of antimicrobial peptides, endogenously produced antibiotics. These small molecules inhibit a wide spectrum of microbial organisms such as bacteria, viruses, fungi, and protozoa and additionally possess potent anti-inflammatory activity facilitating bacterial killing in the absence of significant innate immune stimulation. Antimicrobial peptides in the gastrointestinal tract are produced by epithelial cells and Paneth cells situated at the basis of the small intestinal crypts.

Our work on epithelial antimicrobial peptides led to the characterization of a novel, large family of enteric antimicrobial peptides named *cryptdin-related sequence* (CRS) peptides. Similar to the established large group of enteric α-defensins, CRS peptides are expressed by Paneth cells and are released into the crypt lumen upon proteolytic processing. Expression analysis and representative cloning revealed 17 members of this novel peptide family in intestinal tissue. Synthesized CRS peptides showed broad antibacterial activity and rapid bacterial killing in the absence of detectable eukaryotic cytotoxicity. Mass spectrometric analysis demonstrated the presence of covalent dimeric peptides in various homo-, and heterodimeric combinations *in vivo*. CRS peptides are the first family to be shown to form covalent dimers, a strategy that might significantly expand the antimicrobial spectrum of the intestinal host defence (Hornef *et al.;* 2004).

Recent results suggest that homeostatic epithelial innate immune signalling in the adult host supports the development of enteric Paneth cells and the production of enteric antimicrobial peptides (Stockinger et al., 2014a). Furthermore, secretion by Paneth cells is induced by the parasympaticomimetic acetylcholine and Nod2 ligand muramyl-dipeptide (MDP). In addition, we demonstrated that also endogenous mediators such as IL-4 and IL-13 directly act on Paneth cells and induce degranulation and the secretion of antimicrobial peptides (Stockinger et al., 2014b).

Secreted antimicrobial peptides appear not to diffuse into the intestinal lumen but rather remain attached to the intestinal mucus layer as illustrated by the presence of antimicrobial peptides within isolated mucus material and the high mucus associated antibacterial activity (Meyer-Hoffert *et al.,* Gut, 2008). The presence of antimicrobial peptides within the mucus layer generates a physicochemical shield and restricts bacterial penetration and innate immune stimulation by microbiota-derived immunostimulatory molecules (Dupont et al., 2014).

The neonate intestinal epithelium in mice lacks the formation of intestinal crypts and consequently also the presence of Paneth cells. In contrast, neonatal intestinal epithelial cells produce the *cathelicidin related antimicrobial peptide* (CRAMP). CRAMP expression wanes with the emergence of crypt Paneth cells two weeks after birth and the production of Paneth cell-derived antimicrobial peptides such as CRS peptides and α-defensins (Menard *et al.*, J. Exp. Med., 2008). This postnatal

switch in the antimicrobial repertoire might support the establishment of a stable enteric microbiota after birth.

Current work on antimicrobial peptides focuses on the establishment of models to study the regulatory processes involved in the production, processing, storage, and secretion of these important enteric innate immune effector molecules. These studies aim to define the role of enteric antimicrobial peptides for the maintenance of the normal flora as well as hats defence during infection and may ultimately lead to the development of therapeutic strategies to induce endogenous antimicrobial peptide secretion and prevent or treat enteric microbial infection.

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